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(71) Applicant : **BAYLOR COLLEGE OF MEDICINE**
One Baylor Plaza
Houston, TX 77030 (US)
(71) Applicant : **ABBOTT LABORATORIES**
Abbott Park, Illinois 60064 (US)

(72) Inventor : **Lupski, James R.**
11102 Ashcroft Drive
Houston, Texas 77096 (US)
Inventor : **Katz, Leonard**
844 North Avenue
Waukegan, Illinois 60085 (US)

(74) Representative : **Wise, Stephen James et al**
c/o RAWORTH, MOSS & COOK 36 Sydenham Road
Croydon, Surrey CR0 2EF (GB)

(54) Antisense oligonucleotides complementary to the macromolecular synthesis operon of bacteria, and their uses in diagnosis and therapy.

(57) A method of treating bacterial infections comprising the step of interrupting the expression of a macromolecular synthesis operon in bacteria by hybridizing an antisense oligonucleotide to a single stranded DNA or to a mRNA transcribed from the macromolecular synthesis operon. The antisense oligonucleotide can be either sequence specific to a unique intergenic sequence or a sequence specific to a bacterial homologous sequence. By interrupting the expression of the macromolecular synthesis operon bacterial infections can be treated. Specific antisense oligonucleotides and macromolecular synthesis operon sequences are disclosed. The ability of the antisense oligonucleotide to bind the mRNA or single stranded DNA also allows the identification of the bacteria by using a unique intergenic antisense oligonucleotide to bind to the single stranded DNA or to the mRNA transcribed from the macromolecular synthesis operon. A method for competitively inhibiting the protein products of the MMS operon with oligonucleotides is also disclosed. Methods of identifying unique intergenic sequence is also disclosed.

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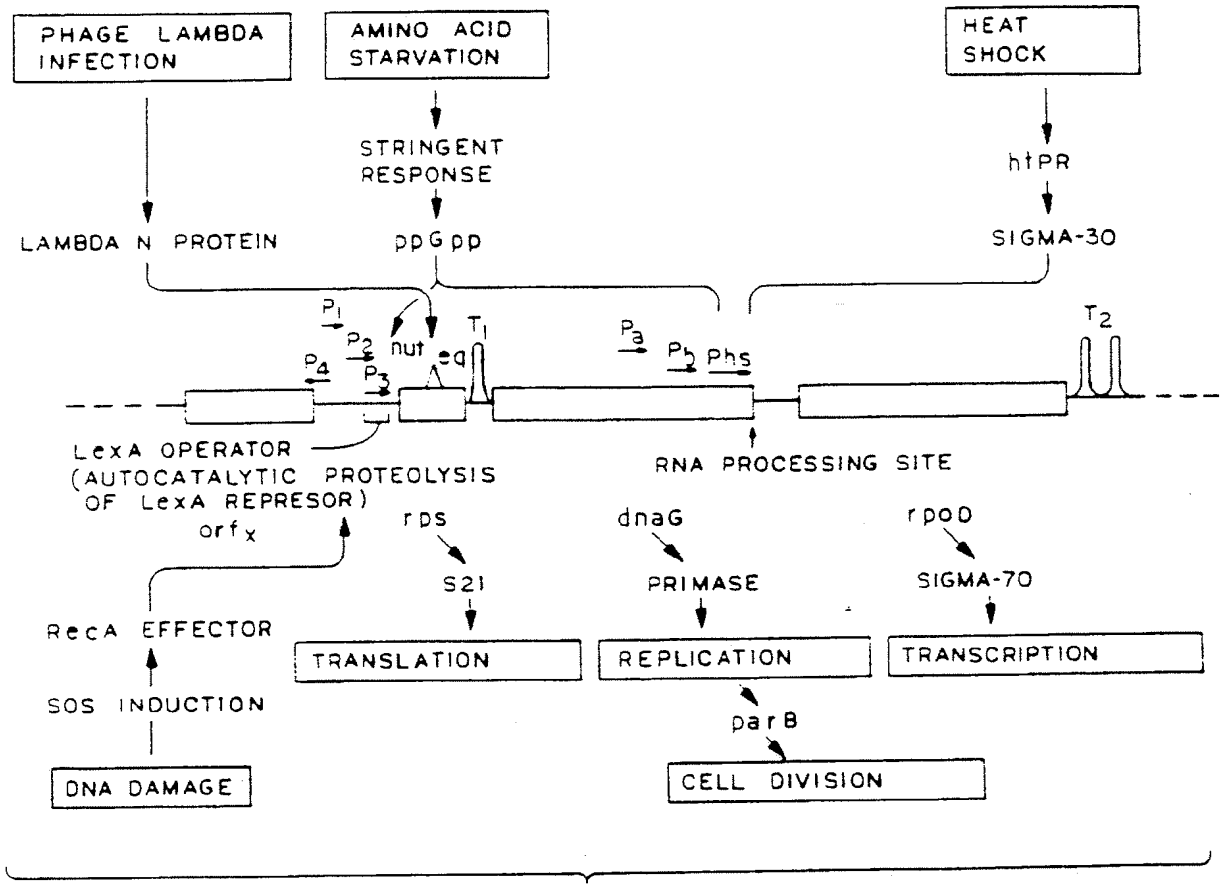

FIG. 2

FIELD OF THE INVENTION

The present invention relates generally to antisense oligonucleotides which bind to a messenger RNA and single strand DNA. More particularly it relates to antisense oligonucleotides which bind to messenger RNA transcribed from the macromolecular synthesis operon of bacteria. It also relates to the treatment of bacterial infections by the introduction of antisense oligonucleotides into bacteria. It further relates to the method of identification of bacteria by the binding of an antisense oligonucleotide specifically to a unique sequence in the intergenic regions of the macromolecular synthesis operon of bacteria. It also relates to the treatment of bacterial infections by competitive inhibition of the macromolecular synthesis operon gene products by utilizing oligonucleotides known to act as recognition sequences for the MMS operon protein products. It also relates to identification of bacteria. It further relates to the isolation and identification of unique intergenic sequences.

BACKGROUND OF THE INVENTION

It has been demonstrated that the genes involved in initiating the synthesis of DNA, RNA and protein in bacteria are contained in one single structural unit named the macromolecular synthesis operon (MMS). The genes are part of a single transcription unit and have been identified as ***rpsU*** encoding ribosomal protein S21 involved in initiating translation, ***dnaG*** encoding the protein primase which initiates DNA replication and ***rpoD*** which encodes sigma-70 involved in initiating transcription. The operon structure is found in both gram negative bacteria, such as ***Escherichia coli*** and ***Salmonella typhimurium***, and in gram positive bacteria such as ***Bacillus subtilis***. The individual structural genes are conserved and have large areas of homology. On the other hand, the intergenic sequences between the structural gene within the operon are unique to each bacterial species. The MMS operon appears to be a central information processing unit for directing the flow of genetic information. The organization of the operon suggests that under certain physiological conditions there is a need for coordination of synthesis of the information macromolecules (DNA, RNA and protein) in the cell and hence a coregulation of the initiator genes. Since the synthesis of each class of macromolecule appears to be regulated at its initiation step, regulation of the MMS operon most likely plays a role in regulating cell growth.

The MMS operon contains three structural genes. The ***rpsU*** gene encodes the ribosomal protein S21 which is required for specific initiation of messenger RNA (mRNA) translation. The protein S21 interacts with a stretch of ribosomal RNA (rRNA) complementary to the mRNA ribosomal binding site called the Shine-Dalgarno sequence located at the 3′ end of the 16S rRNA. Colicin E3 removes 50 nucleotides from the 3′ terminus of 16S rRNA. E3 treated ribosomes cannot carry out polypeptide chain initiation nor chain elongation. In reconstitution experiments, E3 treated ribosomes bind all 30S proteins except S21. RNA protein cross-linking experiments demonstrate that protein S21 is cross-linked to the 3′ dodecanucleotide of the 16S rRNA. The base-pairing potential of the 3′ terminus of 16S rRNA depends on the functional state of the 30S subunit and the presence of S21, which is required for specific initiation of ***E. coli*** and phage MS2 mRNA translation.

Initiation of DNA replication requires a priming RNA which is synthesized by the ***dnaG*** gene product, primase. This protein binds to the phage G4 origin of replication. Primase also is known to interact with the multienzyme complex primosome to initiate synthesis of Okazaki fragments on the chromosomal replication fork-lagging strand of ***E. coli***. Primase is the sole priming enzyme required for initiation of DNA replication at the origin of the ***E. coli*** chromosome. A ***parB*** mutation in the ***dnaG*** gene results in abnormal partition of chromosomes and was originally isolated as a thermosensitive mutant affecting DNA synthesis and cellular division. Thus, in addition to initiation of DNA replication, the ***dnaG*** gene appears to play some role in regulating cell division.

The rpoD gene product sigma-70 is involved in the recognition of promoter sequences for the specific initiation of RNA transcription. Sigma-70 interacts with the core polymerase $\alpha_2\beta\beta'$ conferring specificity for promoter sequences. Sigma-70 is a member of a large family of RNA polymerase sigma factors. Thus, the macromolecular synthesis operon gene products share a common mechanism. Through protein-nucleic acid interactions the gene products of the MMS operon bind specific nucleotide sequences. For example S21 binds the Shine-Dalgarno sequence/ribosome binding site, primase binds the origin of replication, and sigma-70 binds a promoter sequence. These interactions result in initiation of synthesis of protein, DNA or RNA respectively.

Antisense RNAs have been utilized both in nature and experimentally to regulate gene expression. For example antisense RNA is important in plasmid DNA copy number control, in development of bacteriophage P22. Antisense RNAs have been used experimentally to specifically inhibit in vitro translation of mRNA coding from Drosophila hsp23, to inhibit Rous sarcoma virus replication and to inhibit 3T3 cell proliferation when directed toward the oncogene c-fos. Furthermore, it is not necessary to use the entire antisense mRNA since a short antisense oligonucleotide can inhibit gene expression. This is seen in the inhibition of chloramphenicol acetyltransferase gene expression and in the inhibition of specific antiviral activity to vesicular stomatitus virus by

inhibiting the N protein initiation site. Antisense oligonucleotides to the c-myc onocogene have been demonstrated to inhibit entry into the S phase but not the progress from G_0 to G_1. Finally, inhibition of cellular proliferation has been demonstrated by the use of antisense oligodeorynucleotides to PCNA cyclin.

Antibiotics are important pharmaceuticals for the treatment of infectious diseases in a variety of animals including man. The tremendous utility and efficacy of antibiotics results from the interruption of bacterial (prokaryotic) cell growth with minimal damage or side effects to the eukaryotic host harboring the pathogenic organisms. All antibiotics destroy bacteria by interfering with the normal flow of genetic information. This is performed by inhibition of any one of the following: DNA replication, that is, DNA to DNA (for example, the drugs Novobiocin and Nalidixic acid); transcription, that is, DNA to RNA (for example, Rifampin); translation, that is, RNA to protein (for example, tetracyclines, erythromycin and kanamycin); or cell wall synthesis (for example, penicillins).

The present invention provides a new class of antibiotics and a method for the treatment of bacterial infections either generally or specifically. The antibiotics are antisense oligonucleotide sequences which bind mRNA transcribed from the MMS operon. This is a new method of treating bacterial infections by interfering with the fundamental structural unit that regulates the growth and replication of bacteria.

SUMMARY OF THE INVENTION

The present invention seeks to provide a method for the treatment of bacterial infections.

The present invention also seeks to use antisense oligonucleotides to treat bacterial infections.

Further the present invention seeks to provide a method for identifying bacteria.

Still further the present invention seeks to provide a sequence which detects the presence or absence of bacteria

Further still the present invention seeks to provide a method of determining unique intergenic sequences in the macromolecular synthesis operon.

In accordance with one aspect of the present invention there is the method for treating bacterial infections by interrupting the expression of the macromolecular synthesis operon by binding an antisense oligonucleotide antibiotic to a mRNA transcribed from the macromolecular synthesis operon.

In preferred embodiments, the antisense oligonucleotide antibiotic can be selected from the following sequences:

5′ CAITGCTTTGGITGIGGIGCGIIIGGCAA 3′

5′ TTGCCIIICGCICCICAICCAAAGCAITG 3′

5′ CANTGCTTTGGNTGNGGNGCGNNNGGCAA 3′

5' TTGCCNNNCGCNCCNCANCCAAAGCANTG 3',

5' ACITAIGCIACITGGTGGATGIGICAGGC 3',

5' ACNTANGCNACNTGGTGGATCNGNCAGGC 3',

5' GCCTGICIGATCCACCAIGTIGCITAIGT 3',

5' GCCTGNCNGATCCACCANGTNGCNTANGT 3',

5' TTIGCTTCGATITGICGIATACG 3',

5' TTNGCTTCGATNTGNCGNATACG 3',

5' ACGAGCCGTTCGACGTAGCTCTGCG 3',

5' CGGCGTGCGTTTTCGCGAGCCAGT 3',

5' ACATGCCGGTAATTAAAGTACGTG 3',

Another aspect of the present invention is a method for typing or identifying bacteria comprising the steps of binding a unique intergenic antisense oligonucleotide to a mRNA transcribed from the macromolecular synthesis operon or the macromolecular synthesis operon DNA and then determining the amount of binding between the species specific macromolecular synthesis oligonucleotide and the mRNA transcribed from the macromolecular synthesis operon of a given bacterial species.

A further aspect of the present invention is the use of a homologous sequence to detect the presence or

absence of bacteria.

In the treatment of a bacterial infection or in the identification of bacteria the antisense oligonucleotide is at least 10 nucleotides (10 mer). In a preferred embodiment, an oligonucleotide of 16 to 29 mer is used.

An additional aspect of the present invention is the provision of an antisense oligonucleotide antibiotic of at least 10 nucleotides, wherein said oligonucleotide binds to a mRNA transcribed from a macromolecular synthesis operon. In one embodiment the antibiotic further comprises a carrier molecule linked to the oligonucleotide for facilitating the uptake of the oligonucleotide into the bacterium. The carrier molecule can be an amino acid, and in one preferred embodiment is leucine. In another embodiment the 3′ and/or 5′ termini of the oligonucleotide is derivatized to prevent the degradation, e.g. by exonucleases, of the oligonucleotide after bacteria uptake.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is the macromolecular synthesis operon shown in schematic form. It contains three genes, one each, involved in the initiation of translation (***rpsU***), replication (***dnaG***) and transcription (***rpoD***).

Figure 2 depicts the regulation of the ***E. coli*** macromolecular synthesis operon. The three genes in the macromolecular synthesis operon are depicted as closed boxes. The cis-acting regulatory sequences include promoters (P_x, P_1, P_2, P_3, P_a, P_b, P_{hs}) terminators (T_1 and T_2), a LexA binding site, nut_{eq} and an RNA processing site. The ***trans*** acting factors are shown with arrows drawn to where they are believed to act. The NusA protein increases rpoD gene expression, but its site of action is unknown. Global regulatory networks that interact with the macromolecular synthesis operon include the SOS, heat shock and stringent response. A functional role for orf_x has not been assigned, but the proximity of P_x and the conservation of the orf_x sequences in ***E. coli*** and ***S. typhimurium*** suggests a possible macromolecular synthesis operon regulatory role. There are several other open reading frames further upstream with no assigned function and the nearest gene mapped on the ***E. coli*** chromosome is the ***cca*** gene which is 14 kb away.

Figure 3 is a comparison of the macromolecular synthesis operon in different species, ***E. coli, S. typhimurium*** and ***B. subtilis***. The genes are depicted by open boxes with the size given in base pairs (bp) including termination codon. The size of the intergenic sequences is given below. Position of promoters (P) are denoted. AOAMMS - Eco is complementary to the ***E. coli*** macromolecular synthesis operon ***rpsU-dnaG*** intergenic sequences. AOAMMS - Sty is complementary to the ***S. typhimurium*** macromolecular synthesis operon ***rpsU-dnaG*** intergenic sequences. AOAMMS - Bsu is complementary to the ***B. subtilis*** macromolecular synthesis operon P23-***dnaE*** intergenic sequences.

Figure 4 shows a 5′ modified antisense oligonucleotide antibiotic containing the addition of leucine.

Figure 5 shows a 3′ modified antisense oligonucleotide antibiotic.

Figure 6 shows the homologies between bacterial strains for the primase gene. The information was generated from DNA sequences in GenBank utilizing the Molecular Biology Information Resources Multialign program to optimize homology searches of protein sequence data. The data is aligned from left to right on the abscissa, the amino terminal to the carboxy terminal portions of the protein. The numbers represent the amino acid positions in the protein primary sequence. In (a) ***B. subtilis*** was compared to ***E. coli***, while in (b) ***S. typhimurium*** was compared to ***E. coli***, and in (c) ***B. subtilis*** is compared to ***S. typhimurium***. In (d), the ***S. typhimurium*** and ***B. subtilis*** primase protein sequences have been aligned to the ***E. coli dnaG*** primase in the amino terminal region. Upper case letters represent aligned non-identical amino acids while lower case letters signify non-aligned amino acids. The dashes represent aligned identical bases while the dots signify gaps. The data demonstrate that the primase proteins are related and share homology domains particularly in the amino terminal regions. The nucleotide sequence encoding these areas of amino acid homology are also very homologous.

Figure 7 is a picture of 1% agarose gel showing antisense binding of MMS operon probe sequences to restriction digested purified chromosomal DNA by Southern blotting.

Figure 8 is a dot matrix plot of the ***L. monocytogenes rpoD*** amino terminus versus the known sigma factor genes in ***B. subtilis, E. coli*** and ***S. Typhimurium***. The diagonal line represents homologous region shared by the expressed ***rpoD*** sequences of these organisms.

Figure 9 is a dot matrix plot of the ***rpoD-dnaG*** intergenic sequence comparisons between ***L. monocytogenes, E. coli, S. typhimurium*** and ***B. Subtilis***. This demonstrates the species specificity of the intergenic region.

Figure 10 is a dot matrix plot comparison of the ***dnaG/E*** primase genes of ***B. subtilis, E. coli*** and ***S. typhimurium***.

Figure 11 is a dot matrix plot of the ***L. monocytogenes dnaG*** internal segment versus the known primase genes of ***S. typhimurium, E. coli***, and ***B. subtilis***. This demonostrates conserved homologous regions in the

dnaG gene of these organisms.

Figure 12 is a schematic representation of PCR primers used to identify the intergenic sequences between the ***dnaG/E*** and ***rpoD*** genes in the macromolecular synthesis operon.

Figure 13 is a schematic representation of the PCR amplification of the conserved macromolecular synthesis operon regions showing the actual sequence used in the primers. Ec refers to ***E. coli*** primer, St to the ***S. typhimurium*** primer, Bs to the ***B. subtilis*** primer, D refers to the degenerate primer and I refers to the primer with inosine. Conserved homologous region primers were chosen from regions where the amino acid sequence was conserved between ***E. coli, S. typhimurium*** and ***B. subtilis.***

Figure 14 is a schematic representation as well as a gel demonstrating the specificity of the macromolecular synthesis operon intergenic sequences.

Figure 15 is a gel showing that the unique intergenic oligonucleotide probe to the ***E. coli*** macromolecular synthesis operon intergenic regions recognizes all strains within an individual species of ***E. coli.***

Figure 16 is a gel showing a probe to the macromolecular synthesis operon intergenic regions can be utilized to recognize all strains within an individual species of ***S. typhimurium.***

Figure 17 is a gel showing interspecies conservation of the macromolecular synthesis operon sequences among a wide variety of bacterial species

Figure 18 is a gel showing the interspecies conservation of the ***rpoD*** homologous sequences.

Figure 19 is a gel showing the use of probes to the homologous sequence of ***dnaG*** and ***rpoD*** to isolate and identify the ***dnaG-rpoD*** intergenic sequences of various bacteria species.

Figure 20 is a gel showing the use of probe to the homologous sequences of ***rpsU*** and ***dnaG*** to isolate and identify ***rpsU-dnaG*** intergenic sequences in a variety of species.

The drawings are not necessarily to scale and certain features of the invention may be exaggerated in scale or shown in schematic form in the interest of clarity and conciseness.

DETAILED DESCRIPTION

It will be readily apparent to one skilled in the art that various substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

The ***rpsU-dnaG-rpoD*** macromolecular synthesis operon (MMS) is conserved throughout different bacterial species. Further, the gene order and organization of the operon is conserved in all bacteria.

The MMS operon includes genes involved in initiating: translation, ***rpsU***; replication, ***dnaG***; and transcription, ***rpoD***. These genes are contained within a single transcriptional unit, Figures 1 and 2, and are involved in initiating synthesis of the major information macromolecules of the cell: DNA, RNA and protein. The organization of the operon suggests that under certain physiological conditions there is a need for coordination of synthesis of DNA, RNA and protein in the cell and hence a coregulation of the initiator genes. Since the synthesis of each class of information macromolecule (DNA, RNA and protein) appears to be regulated at its initiation step, regulation of the MMS operon most likely plays a role in regulating cell growth.

In the MMS operon ***cis***-acting regulatory sequences can occur within the coding regions. In gram-negative bacteria these include the nut_{eq} site within the ***rpsU*** structural gene and promoters P_a, P_b, and P_{hs} in the ***dnaG*** structural gene. Promoter P_3 of the ***B. subtilis*** MMS operon is within the gene coding for P23. Other cis-acting regulatory sequences are located in the intergenic regions; terminator T_1 is located between ***rpsU*** and ***dnaG*** and an RNA processing site occurs in the ***dnaG-rpoD*** intergenic sequences. Thus, multiple ***cis***-acting regulatory sequences allow discoordinate regulation as well as differential relative rates of individual gene expression within this operon structure.

Codon usage can affect relative amounts of individual gene expression. The presence of codon preference reflects the relative concentrations of isoaccepting tRNA species in the cell. The use of rare codons provides a means to ensure low level expression of regulatory genes. The ***dnaG*** gene contains greater than ten times the number of rare triplet codons as other ***E. coli*** genes and the absolute number of rare codons in the ***dnaG*** mRNA is similar to that of other control genes (e.g. ***lacI***, ***trpR***). Rare codons also occur in the ***S. typhimurium dnaG*** mRNA and the ***dnaE*** gene of ***B. subtilis***. The ***dnaE*** gene is equivalent to the ***dnaG*** gene, each encodes the primase protein which initiates DNA replication. An additional translational regulatory mechanism operative in the MMS operon relies on the occurrence of ribosome binding sites with varying degrees of complementarity to the Shine-Dalgarno sequence. This can be seen in the ***E. coli dnaG*** gene, and is presumably due to the difference in free energy of binding leading to less efficient binding of the ribosome to the ***dnaG*** portion of the MMS mRNA. Both of these translational regulatory mechanisms, rare codon usage and altered ribosome binding affinity may partially explain the observed apparent discoordination of expression of the genes in this operon. The steady state relative abundances for the MMS operon protein products in the ***E. coli*** cell are 40,000 for S21, 50 for primase and approximately 3000 for sigma-70.

Comparative analysis of three sequenced MMS operons reveals several interesting features (Figure 3). All of the operons contain three open reading frames and transcription of the operon is initiated by several promoters at the 5′ end. The major promoters have overlapping nucleotide sequences (-10 and -35 regions) and the ***cis***-acting regulatory sequences appear to be clustered in small regions. Each operon contains a heat shock promoter (p_{hs}) within the DNA replication initiation gene, ***dnaG*** or ***dnaE***. The ***E. coli*** and ***S. typhimurium*** operons contain an open reading frame (orf_x) upstream of the external promoters (P_1, P_2, P_3). Only 7 bp separate the -35 sequences of P_x and P_1 in ***E. coli*** while these sequences actually overlap in the ***S. typhimurium*** operon.

The central gene in the MMS operon is the one involved in initiating DNA replication. The ***dnaG*** gene product, primase has several activities which include (i) a protein-protein interaction with the primosome complex, (ii) a protein-nucleic acid interaction for recognition of the origin, (iii) an RNA polymerase activity to synthesize the primer RNA and (iv) a role in the partitioning of chromosomes as suggested by the ***parB*** mutation in the ***dnaG*** gene. There are no promoters which transcribe the ***dnaG*** gene directly. A 5′ transcription terminator, poor ribosome binding site, occurrence of rare codons and clustering of rare codons are all mechanisms that maintain low level expression of this gene. Overexpression of the ***dnaG*** gene from a regulated promoter on an autonomously replicating plasmid kills the host cells. Evidence that regulation of ***dnaG*** expression directly affects cell growth comes from Tn***5*** mutagenesis data. A cloned ***dnaG*** gene with the MMS operon promoters intact, on a multicopy plasmid slows the growth rate of the host cell harboring it. After insertion of Tn***5*** into the ***dnaG*** promoter regions, presumably leading to decreased ***dnaG*** gene expression, growth rates return to control levels demonstrating that an increased ***dnaG*** expression can affect growth. Isolation of the ***parB*** mutation also suggests a direct role for ***dnaG*** in chromosome partitioning, cell division, and therefore, bacterial cell growth. The primase proteins encoded by the DNA replication initiation genes from the three Sequenced MMS operons contain several regions of homology (Figure 6).

The MMS operon is under very complex regulatory control which, teleologically would be expected of a unit whose control is important to regulation of cell growth. In addition to the intrinsic complex regulation, the operon interacts with several global regulatory networks including heat shock, the stringent response, and SOS. This operon appears to have evolved ways to be regulated both as a single unit and as a group of independent units by strategic positioning of transcriptional and translational control signals. The fact that the operon is the same in ***E. coli*** and ***S. typhimurium*** and very similar in ***B. subtilis*** suggests there is a selective advantage to evolving such a structure.

The term "oligonucleotide" as used herein, defines a molecule comprised of more than three deoxyribonucleotides or ribonucleotides. Its exact length will depend on many factors relating to the ultimate function or use of the oligonucleotide. A fragment of a sequence is any molecule containing some smaller part of a larger molecule. A derivative of the molecule includes alterations or additions to the 3′ or 5′ termini substitution of a base by inosine or a degenerate code substitution.

The term "homologous sequence" as used herein, defines a sequence within the MMS operon which has been conserved in bacterial species such that the sequence is nearly identical among a variety of species. Thus, because of its homology, this sequence cannot be used to distinguish different types of bacteria from themselves. However, this sequence can be used to determine the presence or absence of bacteria or as a target to attack with a single agent and thus interfere with the MMS operon expression in a variety of bacterial species.

The term "unique intergenic sequences" as used herein, defines a section of non-coding DNA which is positioned between the ***rpsU-dnaG/E*** and ***dnaG/E-rpoD*** genes within the MMS operon. In Figure 3 some examples of the location within the MMS operon of the unique intergenic sequences, AOAMMS-Sty, AOAMAS-Eco and AOAMMS-Bsu, are shown. These MMS operon intergenic sequences are unique for each different species of bacteria. Thus, a specific sequence will be characteristic for a specific species of bacteria. Because of this uniqueness, the intergenic sequences can be used to identify the bacteria or for targeting a specific agent to kill or interrupt the functioning of a specific bacteria.

The term "antisense" as used herein, defines an oligonucleotide the sequence of which is complementary to the sense strand of the MMS operon. An antisense oligonucleotide will hybridize or bind (form a complex by Watson-Crick base pairing) in a complementary fashion to the messenger RNA molecule which has been transcribed from the MMS operon, as well as to a single stranded DNA of the MMS operon. The antisense oligonucleotide can be designed to bind to either a unique intergenic sequence, a homologous sequence or a combination of both unique and homologous sequences.

The term "antibiotic" as used herein, means an antisense oligonucleotide capable of interfering with the MMS operon to slow down bacterial growth thereby arresting growth and provoking cell death.

"Derivitizing" the oligonucleotide means altering the structure of the oligonucleotide to perform a specific function (e.g. (1) an addition to the 3′ or 5′ end to increase uptake into the cell; (2) blocking the 3′ or 5′ end to prevent exonucleolytic breakdown). This procedure may provide a more functional and stable oligonucleotide when it is in the bacteria. For example, the 3′ end can be derivitized by adding a phosphorothioate linked nuc-

leotide.

One embodiment of the present invention is a method for treating bacterial infections comprising the step of interrupting the expression of a MMS operon by hybridizing an antibiotic to a mRNA transcribed from said MMS operon. The antibiotic can hybridize to either a homologous sequence, a unique intergenic sequence or a combination thereof.

One skilled in the art readily recognizes that the antisense oligonucleotide can be delivered to the bacteria by a variety of commonly used delivery systems. For example, nasal spray, intravenous or intramuscular or intrathecal injection, oral or suppository administration. The specific choice of method depends on the location of the bacteria and is well within the skill in the art to determine. A small, 10-29 mer, antisense oligonucleotide that is delivered to a bacteria, is rapidly transported into the bacterial cell. Additionally, by modifying the 3′ or 5′ ends of the antisense oligonucleotide the rate of uptake or the specificity of uptake can be adjusted.

The antisense oligonucleotide is selected from the group consisting of a sequence specific to a unique intergenic sequence, a sequence specific to a bacterial homologous expressed sequence and any combination thereof.

By hybridizing to a specific unique intergenic sequence encoded in the single stranded DNA or mRNA which has been transcribed from the MMS operon, the antibiotic is targeted to interrupt and kill a specific type of bacteria. On the other hand, by hybridizing to the homologous sequence, the antibiotic is targeted to kill a wide variety of bacteria, i.e., all bacteria containing the homologous sequence. Depending on the length of the oligonucleotide or the location of the mRNA which is bound, the oligonucleotide may overlap and bind to both a unique sequence and a homologous sequence.

The exact length of the oligonucleotide needed to inhibit the functioning of the mRNA is at least 10 nucleotides (10 mer). In the preferred embodiment of the present invention, the oligonucleotide is in the range of 16 to 29 mer.

Some examples of sequences which are used to bind to the mRNA to interrupt the function of the MMS operon and thus to treat bacterial infections are seen in Tables 1 and 2.

Table 1

Homologous Sequences Which Bind to mRNA Transcribed From the MMS Operon or to Single-Stranded Bacterial DNA Containing the MMS Operon

MMS ALL1I	5' CAITGCTTTGGITGIGGIGCGIIIGGCAA 3'
MMS ALL1I-R	5' TTGCCIIICGCICCICAICCAAAGCAITG 3'
MMS ALL1D	5' CANTGCTTTGGNTGNGGNGCGNNNGGCAA 3'
MMS ALL1D-R	5' TTGCCNNNCGCNCCNCANCCAAAGCANTG 3'
MMS ALL2I	5' ACITAIGCIACITGGTGGATGIGICAGGC 3'
MMS ALL2D	5' ACNTANGCNACNTGGTGGATCNGNCAGGC 3'
MMS ALL3I	5' GCCTGICIGATCCACCAIGTIGCITAIGT 3'
MMS ALL3D	5' GCCTGNCNGATCCACCANGTNGCNTANGT 3'
MSS ALL4I	5' TTIGCTTCGATITGICGIATACG 3'
MMS ALL4D	5' TTNGCTTCGATNTGNCGNATACG 3'
MMS RPSU1	5' ACGAGCCGTTCGACGTAGCTCTGCG 3'
MMS RPSU2	5' CGGCGTGCGTTTTCGCGAGCCAGT 3'
MMS RPSU-5'ATG	5' ACATGCCGGTAATTAAAGTACGTG 3'
(AOAMMS-*dnaG*)	5'CATCCAAAGCAGTGGTAAAACTGTTT 3'
(AOAMMS-*rpoD*)	5'TCACCGATCGGCGTTTCCA 3'

Table 2

Unique Intergenic Sequences Which Bind to mRNA Transcribed from the MMS Operon or to Single-Stranded Bacterial DNA Containing the MMS Operon

Abbreviation	Sequence	Bacterial Sour
MMS BS1	5' GGGATTTGCACTAAAGCATCG 3'	*B. subtilis*
MMS BS2	5' GATCGCTTAACCTCATCATG 3'	*B. subtilis*
AOAMMS-Bsu	5' TATTCGATGCTTTAGTGC 3'	*B. subtilis.*
MMS CHLAM1	5' GTCGGTGTAGGAAGTTTTTCTAGGGCCG 3'	*C. trachomatis*
MMS EC1	5' TTATCGTTGGCGGTAAACAACCGTTGG 3'	*E. coli*
AOAMMS-Eco	5' GGCCCCGATTTTTAGCAA 3'	*E. Coli*
MMS HRDB1	5' CCACGCGGATTGGGCGTAACGCTCTTGGG 3'	*S. coelicolor*
MMS HRDB1-R	5' CCCAAGAGCGTTACGCCCAATCCGCGTGG 3'	*S. coelicolor*
MMS LIST1	5' CGTGTCATGCTCGAAATCGTCCAACTC 3'	*L. monocytogen*
MMS MYXX1	5' CGCCCATGCAACCGGTTTGAGTTCGCG 3'	*M. xanthus*
MMS ST1A	5' CGGCGCTTACGCAAGTCAGCGACA 3'	*S. typhimurium*
MMS ST2B	5' CGACAGCTATACCGTCGACACC 3'	*S. typhimurium*
AOAMMS-Sty	5' CTTGCGTAAGCGCCGGGG 3'	*S. typhimurium*

The sequences in Table 1 bind to bacterial homologous sequences and thus kill a wide variety of bacterial species. These sequences are useful in treating a wide class of bacterial infections, since they attack both gram positive and gram negative bacteria.

The sequences in Table 2 are unique intergenic sequences which bind to specific sequences in specific bacteria. Employing an antisense oligonucleotide from Table 2 as an antibiotic will specifically inhibit the MMS operon of the bacteria for which it is specific, while not attacking the MMS operon of other bacteria. Each sequence in Table 2 is followed by the type of bacteria which is sensitive to the sequence. Employing these unique antisense oligonucleotides uses a specific antibiotic to kill a specific bacteria. Thus, the treatment to kill or interfere with the reproduction of specific bacterial species is targeted.

In the preferred embodiment, using unique sequences, the nucleotide sequence of the proposed antisense oligonucloetide antibiotics is complementary to the intergenic region of the 5′ side of the DNA replication initiation gene (***dnaG*** or ***dnaE***) (see Figure 3). This region of the MMS operon is chosen because the replication initiation gene has the lowest level of expression within the operon. Furthermore, in ***E. coli*** and ***S. typhimurium***, this gene is located downstream from a terminator and is not directly transcribed by any promoter. In order to provide a more stable irteraction with the mRNA, the primary sequences of the antisense oligonucleotide are chosen to maximize GC base pairing. However, one skilled in the art recognizes that there is a balance between maintaining the uniqueness of the sequence and maximizing the GC base pairing.

Another embodiment of the invention is a method of identifying bacteria comprising the steps of hybridizing a known species specific unique intergenic antisense oligonucleotide to a mRNA transcribed from a MMS operon or a single stranded DNA and measuring the amount of said hybridization to determine the type of bacteria. The unique sequence will only hybridize to a specific bacteria species, therefore no hybridization indicates a different species and hybridization indicates the species with the specific sequence. Each bacterial species

contains a MMS operon with a unique intergenic sequence which can be used to uniquely identify each species. The mRNA which is transcribed from the MMS operon spans the whole operon and contains the homologous and unique intergenic sequences. By designing oligonucleotides which bind to the unique intergenic sequences, the diagnosis and treatment can be tailored to only identify and interfere with the functioning of a MMS operon in those bacterial species which have that unique sequence. Thus, by using a variety of antisense oligonucleotide probes, bacteria can be typed for each individual species. The amount of hybridization can be determined by a variety of methods known to those skilled in the art, including radioisotopes, enzymes, fluorescers, antibodies and chemiluminescers For example, the unique species specific intergenic antisense oligonucleotides can be labelled with biotin and then identified by a Strep avidin complex or a fluorescent tag.

The antisense oligonucleotides of Table 2 can be used to identify those bacteria which are listed after each antisense oligonucleotide sequence. One skilled in the art will readily recognize that as additional MMS operon intergenic sequences are sequenced, the present invention can be used to identify additional bacteria by antisense oligonucleotides synthesized to the unique intergenic sequences.

In bacteria typing, the length of the antisense oligonucleotide will be determined by the size necessary to bind specifically to the unique sequence. The oligonucleotide should be at least 10 nucleotides in length. In a preferred embodiment the sequences are between 16 and 29 mer. Examples of some preferred sequences are found in Table 2.

In order for the antisense oligonucleotide antibiotic to effectively interrupt the MMS operon function by hybridizing to the mRNA transcribed from the MMS operon, the antisense oligonucleotide antibiotic must enter the bacterial cell. Although oligonucleotides are taken up by bacterial cells, some modification of the oligonucleotides can help facilitate or regulate uptake. Thus, a carrier molecule, for example an amino acid, can be linked to the oligonucleotide. In Figure 4, the oligonucleotide is modified at the 5′ end by adding a leucine molecule to the oligonucleotide. Bacteria have multiple transport systems for the recognition and uptake of molecules of leucine. The addition of this amino acid to the oligonucleotide facilitates the uptake of the oligonucleotide in the bacteria and does not interfere with the binding of the antisense oligonucleotide to the mRNA molecule.

One skilled in the art will readily recognize that other methods are available for facilitating the uptake of the antisense oligonucleotide antibiotic in the bacteria and for increasing the stability of oligonucleotides once inside the bacteria. For example, addition of other amino acids or peptides or primary amines to the 3′ or 5′ termini enables utilization of specific transport systems and inhibits cellular nuclease attack. Addition of lactose to the oligonucleotide by a covalent linkage can enable transport by lactose permease (product of the ***lac*** operon ***Y*** gene). Other sugar transport systems, known to be functional in bacteria, can be utilized to facilitate uptake into the bacterial cell.

Once an oligonucleotide with or without the carrier has entered the bacterial cell, it is important that it remain stable for the time period necessary !o bind to the mRNA transcribed by the MMS operon. In one embodiment of the present invention, the oligonucleotide is derivatized at the 3′ end to prevent degradation of the oligonucleotide (Figure 5). Other methods are known to alter the 3′ and/or 5′ ends of oligonucleotides to prolong the intracellular life and thus increase the availability for binding to the mRNA. For example, the addition of a primary amine to the 3′ or 5′ termini inhibits exonuclease activity and increases the cell life of antisense oligonucleotides.

The expressed sequences or genes, ***rpsU, dnaG,*** and ***rpoD***, within the MMS operon have regions that are conserved or homologous in all bacteria. These conserved homologous regions are utilized to identify the presence or absence of any bacteria by hybridizing an antisense ologonucleotide that identifies the conserved homologous sequences.

The intergenic regions are DNA sequences between the expressed sequences ***rpsU-dnaG*** and ***dnaG-rpoD***. Intergenic sequences have not been conserved and thus are unique to a given bacterial species. Thus, these unique intergenic sequences are useful in identifying a particular species of bacteria.

Species specific unique intergenic sequences from the macromolecular synthesis operon were obtained from the ***dnaG-rpoD*** or the ***rpsU-dnaG*** regions. Examples of ***homologous*** and ***unique intergenic sequences*** are the ***dnaG, rpoD*** and ***dnaG-rpoD***, respectively, sequences from ***L. monocytogenes*** Tables 3 and 4 and the ***rpsU, dnaG*** and ***rpsU-dnaG,*** respectivly, sequences from ***H. influenzae*** Table 5.

Table 3

L. monocytogenes DNA sequence including the *dnaG* carboxy terminus (numbered 1 to 282), *dnaG-rpoD* intergenic region (numbered 283 to 461) and the *rpoD* amino terminus (numbered 462 to 1043).

```
GCA ACT TCT TGG TGC AAC ATC GTT TAT CAT GAT AAT
Ala Thr Ser Trp Cys Asn Ile Val Tyr His Asp Asn
1               5                  10

TAC AAA GCG CTT TAT ACC TAT CTA ATT GGT TAT TTC
Tyr Lys Ala Leu Tyr Thr Tyr Leu Ile Gly Tyr Phe
        15                  20
```

```
TGG CAG AAG GTA ATG ATG CAG ATC CAA CGG AAA TTT                    108
Trp Gln Lys Val Met Met Gln Ile Gln Arg Lys Phe
25                  30                  35

ATG GAT AGT GTT CCT GAT GCT ACA ATG AAA GGA CTT                    144
Met Asp Ser Val Pro Asp Ala Thr Met Lys Gly Leu
            40                  45

ATC AGT AGC CTC GAA ATG GTT ATT AGT CCA GAT GAA                    180
Ile Ser Ser Leu Glu Met Val Ile Ser Pro Asp Glu
    50                  55                  60

CAA GGT AAA ACA CAG TTT GAA GAC TAT ATT AGA AGT                    216
Gln Gly Lys Thr Gln Phe Glu Asp Tyr Ile Arg Ser
                65                  70

CTA AAG CGG TTT AAA TTA GAA CAA AAG AAA AAA GAA                    252
Leu Lys Arg Phe Lys Leu Glu Gln Lys Lys Lys Glu
        75                  80

CTT GAG CAA GAG CTA AGC AAC TTT AAA TCG                            282
Leu Glu Gln Glu Leu Ser Asn Phe Lys Ser
85                  90

TGAAAATGAC AAAGATAACG AAATTCGTGT CATGCTCGAA                        322

ATCGTCCAAC TCAACCGTCA GTTAAACAGC GGCCAATTGG                        362

ATTAATAACG TTTTAAAACC GCTAAATGAT GGTATTATTA                        402

CCTAAGAGAA GCCTTTTAAT AAGGTTAGCG GCATTTTGGA                        442

AGGAGGAATA CAGGCAGTT                                               461

ATG AGT GAT AAA ACA AAA AAC ACA AAA CCA GTT GCT                    497
MET Ser Asp Lys Thr Lys Asn Thr Lys Pro Val Ala
            5                   10

GAA CTA AGT GTT GAG CAA GTA AAA GAA GCC CTG ATA                    533
Glu Leu Ser Val Glu Gln Val Lys Glu Ala Leu Ile
        15                  20

GAA GAA GGT AAG AAA AAG GGG ATT TTA ACT TAT GCA                    569
Glu Glu Gly Lys Lys Lys Gly Ile Leu Thr Tyr Ala
25                  30                  35

AAA ATC GCT GCC AGA TTA GCT CCA TTC ACT TTG GAT                    605
Lys Ile Ala Ala Arg Leu Ala Pro Phe Thr Leu Asp
            40                  45

TCC GAT CAA ATG GAT GAG TAT TTA GAA CAT GTT GGT                    641
Ser Asp Gln MET Asp Glu Tyr Leu Glu His Val Gly
    50                  55                  60
```

```
GAA GCA GGA ATT GAA GTT TCT GAC GAT GCA GAT GAT                    697
Glu Ala Gly Ile Glu Val Ser Asp Asp Ala Asp Asp
                    65                  70

GAG GAT CCG GAT GAA ACA GAA CTT GTA AAA GAA GAA                    713
Glu Asp Pro Asp Glu Thr Glu Leu Val Lys Glu Glu
        75                  80

ACC GAA TCC TTT GAT TTA ACA GAT ATG AGT GTA CCA                    749
Thr Glu Ser Phe Asp Leu Thr Asp MET Ser Val Pro
85                  90                  95

CCA GGC GTA AAA ATT AAT GAC CCT GTT CGC ATG TAT                    785
Pro Gly Val Lys Ile Asn Asp Pro Val Arg MET Tyr
            100                 105

CTG AAA GAA ATT GGT CGA GTA GAC TTA CTT ACA GCG                    821
Leu Lys Glu Ile Gly Arg Val Asp Leu Leu Thr Ala
    110                 115                 120

GAT GAA GAA ATT GCC TTA GCA AAA CGT ATC GAA GCT                    857
Asp Glu Glu Ile Ala Leu Ala Lys Arg Ile Glu Ala
                125                 130

GGC GAC ATT GAA GCC AAA GGA CGT CTT GCA GAA GCC                    893
Gly Asp Ile Glu Ala Lys Gly Arg Leu Ala Glu Ala
        135                 140

AAC CTG CGC CTT GTT GTA AGT ATT GCA AAA CGT TAT                    929
Asn Leu Arg Leu Val Val Ser Ile Ala Lys Arg Tyr
145                 150                 155

GTT GGT CGC GGT ATG TTA TTC CTT GAT TTA ATT CAA                    965
Val Gly Arg Gly MET Leu Phe Leu Asp Leu Ile Gln
            160                 165

GAA GGT AAC ATG GGA CTA ATG AAA GCC GTT GAG AAA                   1001
Glu Gly Asn MET Gly Leu MET Lys Ala Val Glu Lys
    170                 175                 180

TTC GAC TTC AAT AAA GGA TTT AAA TTC AGT ACC TAT                   1037
Phe Asp Phe Asn Lys Gly Phe Lys Phe Ser Thr Tyr
                185                 190

GCA ACG                                                          1043
Ala Thr
```

Table 4

L. monocytogenes DNA sequence of an internal segment of *dnaG*.

```
A AGC TTA ACG GAA GAA CAT GCA GAT TTA ATT AAA CGG      37
  Ser Leu Thr Glu Glu His Ala Asp Leu Ile Lys Arg
  1               5                  10

CTT ACT AAC CGG GCG ATT ATT TGT TAT GAC GGT GAC      73
Leu Thr Asn Arg Ala Ile Ile Cys Tyr Asp Gly Asp
        15                  20

AGA GCC GGA ATT GAA GCA GCC TAT AAG GCG GGC ACG     109
Arg Ala Gly Ile Glu Ala Ala Tyr Lys Ala Gly Thr
25                  30                  35

CTT CTA GTT GAA CGG AAT CGT TTA GAT GTT TTT GTT     145
Leu Leu Val Glu Arg Asn Arg Leu Asp Val Phe Val
            40                  45

TTG CAA CTT CCA GCT GGA AAA GAT CCC GAT GAC TTT     181
Leu Gln Leu Pro Ala Gly Lys Asp Pro Asp Asp Phe
    50                  55                  60

ATT CGA GCA AGT GGT CCA GAA AAA TTC AAA GAA GTT     217
Ile Arg Ala Ser Gly Pro Glu Lys Phe Lys Glu Val
                65                  70

TAT AAG CAA CAA CGA TCG ACT TGG ACA GCT TTT AAA     253
Tyr Lys Gln Gln Arg Ser Thr Trp Thr Ala Phe Lys
        75                  80

TTC ATT ATT TAC GTA GAG AAC GTA                     277
Phe Ile Ile Tyr Val Glu Asn Val
85                  90
```

Table 5

H. Influenzae. DNA sequence including the *rpsU* gene (numbered 1 to 213), the *rpsU-dnaG* intergenic region (numbered 214 to 350) and the *dnaG* gene (numbered 351 to 548).

```
ATG CCG GTA ATT AAA GTA CGT CAA AAC GAA TCA TTT      36
Met Pro Val Ile Lys Val Arg Glu Asn Glu Ser Phe

GAC GTA GCT TTA CGT CGT TTC AAA CGC TCT TGC GAA      72
Asp Val ala Leu Arg Arg Phe Lys Arg Ser Cys Glu
```

0793G/A

```
AAA GCG GGA ATC TTA GCT GAA ATA CGC GCT CGC GAA          108
Lys Ala Gly Ile Leu Ala glu Ile Arg Ala Arg Glu

TTT TAC GAA AAA CCA ACT ACA ATT CGT AAA CGT GAA          144
Phe Tyr Glu Lys Pro Thr Thr Ile Arg Lys Arg Glu

AAT GCA ACA CTT GCA AAA CGT CAC GCA AAA CGC AAC          180
Asn Ala Thr Leu Ala Lys Arg His Ala Lys Arg Asn

GCT CGC GAA AAC GCG CGC AAT ACC CGT TTA TAC              213
Ala Arg Glu Asn Ala Arg Asn Thr Arg Leu Tyr

TAATTTATAG TATTTTCTGA CTCGAGTTAA GACAAACCGT              253

GAATCCTTTG GACTCACGGT TTTGTTACTT TAAGGCACAA              293

CAAAAATCTA CGCCAAAAAC GACCGCACTT TCACACCACG              333

ATCACGGAGG CTCGACA ATG AAA GGT TCT ATT CCA CGC           371
                   Met Lys Gly Ser Ile Pro Arg

CCC TTT ATT GAT GAT TTG CTG ACA AAG TCC GAT ATT          407
Pro Phe Ile Asp Asp Leu Leu Thr Lys Ser Asp Ile

GTC GAT GTG ATT AAC ACG CGC GTA AAA CTA AAA AAA          443
Val Asp Val Ile Asn Thr Arg Vel Lys Leu Lys Lys

GCT GGC CGC GAT TAT CAA GCC TGC TGC CCT TTC CAT          479
Ala Gly Arg Asp Tyr Gln Ala Cys Cys Pro Phe His

CAC GAA AAA ACA CCA TCC TTC ACA GTT AGC CAA AAG          515
His Glu Lys Thr Pro Ser Phe Thr Val Ser Gln Lys

AAA CAG TTT TAT CAC TGC TTT GGC TGC GGC GCG              548
Lys Gln Phe Tyr His Cys Phe Gly Cys Gly Ala
```

In addition to interrupting the MMS operon by binding to the mRNA transcribed from the operon, it is also possible to control other downstream products of the MMS operon to interrupt bacterial growth and to treat bacterial infections. For example, interrupting the function of the proteins encoded in the MMS operon also interrupts the function of the MMS operon and leads to death of the bacteria.

One embodiment of the present invention is a method for treating bacterial infections comprising the step of interrupting the function of proteins selected from the group consisting of S21, primase and sigma-70. This method comprises the step of competitively inhibiting a recognition site of a protein encoded by the MMS operon by introducing a competitive oligonucleotide into the bacteria.

The S21 recognition site includes the Shine-Dalagarno sequence located at the 3′ end of the 16S rRNA and may be inhibited by introducing an oligonucleotide which competitively inhibits the binding of S21 in the bacteria. For example, an oligonucleotide of the sequence

5' GATCACCTCCTTA 3'.

The primase recognition site includes the phage G4 origin of replication site. Thus by introducing into bacteria a competitive oligonucleotide which interferes with this recognition site, bacterial growth and survival may be inhibited. An example of this competitive inhibitor is the loop III of the bacteriophage G4 ori_c

5'GGCCGCCCCACATTGGGCAGGTATCTGACCAGTAGAGGGGCGGCC 3'.

The sigma-70 recognition site includes the core polymerase $\alpha_2\beta\beta'$ and this interaction confers specificity for promoter sequences. An example of this competitive inhibitor is

5 ' TTGACATAAATACCACTGGCGGTGATACT 3 ' .

This sequence is the bacteriophase lambda P_L promoter. This is the strongest promoter in E. coli and thus has the strongest known binding with RNA polymerase.

Thus the introduction of competitive oligonucleotides for these sequences into the bacteria will result in competitive interaction with the protein recognition site, thus preventing the binding of the S21, primase or sigma-70 molecules to the recognition site. This will interrupt normal cell function, growth and replication. Introduction of these oligonucleotides into the bacteria, disrupts the MMS operon's function and thus successfully treats bacterial infections.

The following examples are offered by way of illustration and are not intended to limit the invention in any manner.

Example 1

The MMS Operon

The positions within the MMS operon of the primers used in the following examples are depicted in Figure 12, and the sequences are shown in Tables 3 and 4. At the top of Figure 12 is a schematic representation of a portion of the general schema for all MMS operons. The expressed sequences or genes dnaG and rpoD are depicted by hatched boxes. The conserved areas within the genes are depicted by arrows. Intergenic sequences are depicted by a single line between hatched boxes. The point of the arrow represents the 3′ end of the individual primers. Nomenclature for actual DNA sequences of each primer is as follows: (1) MMS ALL - refers to primers or probes which recognize homologous regions of the MMS operons from all bacteria. They are based on conserved regions of the ***dnaG*** gene and ***rpoD*** gene in these organisms. MMS ALL#I refers to oligonucleotides where inosine is used to allow base pairing at nonconserved nucleotides while MMS ALL#D represents a degenerate oligonucleotide wherein any base is a possible replacement in the nonconserved position. The individual # refers to the position within the MMS operon. MMS Ec#, MMS St#, and MMS Bs# refers respectively to ***Escherichia coli, Salmonella typhimurium*** or ***Bacillus subtilis*** species specific identifier sequences from the MMS operon intergenic regions. The primers used can be:

(1) primers with the actual sequence; (2) primers which contain inosine substitutions; and (3) combinations of degenerate primers.

In Figure 2 and Figure 3, the actual nucleotide sequences, or composition of matter, used in the experiments are shown.

Example 2

Isolation of Unique Intergenic Sequences From the MMS Operon of ***L. monocytogenes***

The conserved homologous regions from the expressed genes within the MMS operon were used to obtain the nucleotide sequence of the unique intergenic species specific regions of the MMS operon. Oligonucleotide primers complementary to the conserved homologous region from the ***rpsU, dnaG*** and ***rpoD*** genes were made. Combinations of these primers were used in a polymerase chain reaction (PCR) on bacterial chromosomal DNA from diverse bacterial species. Almost all bacteria amplified a specific unique DNA fragment from DNA located in the MMS operon. This unique DNA sequence was located in the intergenic region between the primers and contains the unique intergenic sequence.

In ***L. monocytogenes*** the MMS operon DNA sequences were amplified using primers to the conserved homologous regions of ***dnaG*** and ***rpoD***. The primer to the ***dnaG*** gene is 5′ to 3′ and complementary to the 3′ to 5′ strand, while the primer to the ***rpoD*** gene is 3′ to 5′ and complementary to the 5′ to 3′ strand. The PCR amplified fragment was sequenced to determine the entire DNA sequence. This sequence was compared with published sequences of the ***dnaG/E*** and ***rpoD*** genes from ***E. coli***, ***S. typhimurium*** and ***B. subtilis***. (Figures 8, 10 and 11). It was readily apparent that the PCR amplified sequence from ***L. monocytogenes*** correspond to the ***dnaG/E*** and ***rpoD*** expressed genes from other bacterial species. From comparing the sequences the ***dnaG*** and ***rpoD*** intergenic region for ***L. monocytogenes*** was deduced. (Figures 8, 9 and 11). The DNA sequence in this 197 bp ***L. monocytogenes dnaG*** and ***rpoD*** intergenic region is unique to the ***Listeria*** species. More importantly, computer analysis by dot plot matrix demonstrates that these ***Listeria dnaG-rpoD*** intergenic sequences do not share homology with the ***dnaG-rpoD*** intergenic regions from ***E. coli, S. typhimurium*** or ***B. subtilis***. (Figure 9). This approach of using PCR amplification from the conserved homologous regions and computer comparisons of the amplified sequence by dot matrix plots with known MMS operon is a new and

unique approach to isolating intergenic sequence. One skilled in the art will readily apPreciate the applicability of this technique across a wide spectrum of bacteria.

Example 3

Isolation of Unique Intergenic Sequence from the MMS Operon of ***H. influenzae***

In ***H. influenzae*** the conserved homologous regions from the expressed genes, ***rpsU*** and ***dnaG*** genes were used as primers to amplify the macromolecular synthesis operon ***rpsU-dnaG*** intergenic sequences from ***H. influenzae***. The primer to the ***rpsU*** gene is 5′ to 3′ and complementary to the 3′ to 5′ strand while the primer to the ***dnaG*** gene is 3′ to 5′ and complementary to the 5′ to 3′ strand. The PCR amplified fragment was sequenced to determine the entire DNA sequence. The sequence was compared with the published sequences of the ***rpsU*** and ***dnaG/E*** genes from ***E. coli, S. typhimurium*** and ***B. subtilis***. It was readily apparent from the analysis of the PCR amplified sequence from ***H. influenzae***, which regions corresponded to the ***rpsU*** and ***dnaG/E*** expressed genes. This enabled the deduction of the ***rpsU-dnaG*** intergenic region for ***H. influenzae***.

The data from ***L. monocytogenes*** and ***H. influenzae*** clearly show that oligonucleotides complementary to the conserved regions in the expressed sequences of the macromolecular synthesis operon can be used as primers in a PCR reaction with chromosomal genomic DNA from any bacterial species to identify unique intergenic sequences.

Example 4

Conserved Sequences within the MMS operon

To show that the expressed sequences within the MMS operon, ***rpsU, dnaG, rpoD***, contain conserved homologous DNA sequences, the following oligonucleotide which recognized conserved DNA sequences within the ***dnaG*** gene was synthesized:

5′-CATCCAAAGCAGTGGTAAAACTGTTT-3′.

This oligonucleotide was end labeled and used as a probe in Southern blotting. DNA was isolated from 12 different pathogenic strains of ***Salmonella*** obtained from the body fluids of infected patients, digested with ***Hin-d*III** and run on a 1% agarose gel. This digested chromosomal DNA was probed with the end-labeled ***dnaG*** oligonucleotide AOAMMS.

As seen in Figure 7, there is conservation of the oligonucleotide AOAMMS - ***dnaG*** in different pathogenic strains of ***Salmonella.*** The Southern blot shows homology of the oligonucleotide AOAMMS-***dnaG*** to a laboratory control strain of Salmonella (LT-2) (lane 1) and twelve (12) different pathogenic strains isolated from body fluids of patients (lanes 2-13). There was no hybridization to human DNA (the negative control on lane 14), and as a positive control; a plasmid containing the DNA sequences in the probe showed a hybridization signal (lane 16). Lane 15 has lambda DNA cut with ***Hind*** III as a marker. On the far right are the sizes in kilobase pairs as determined on the agarose gel before Southern transfer.

Example 5

Inhibition of Cell Growth

To inhibit cell growth, an inoculum of ***E. coli*** and ***B. subtilis*** are mixed in a single test tube and an antisense oligonucleotide to ***E. coli*** (AOAMMS-Eco) is added to the cell inoculum. The culture is gram stained after several hours of growth. Gram positive organisms are seen and there is a paucity of gram negative organisms. In a corollary experiment, an antisense oligonucleotide to ***B. subtilis*** (AOAMMS-Bsu) is added to a mixed inoculum of ***E. coli*** and ***B. subtilis*** and it is grown for several hours. On subsequent gram stain there is found negative rods. These experiments demonstrate species specific antisense oligonucleotide demise of bacterial organisms.

Example 6

Hybridization of Probes

For the identification examples herein, a variety of methods can be used to identify bacteria using the unique intergenic sequence or the homologous sequence. For example: (1) the probe can be hybridized to the intergenic sequence directly as RNA or single stranded DNA in the bacteria; or (2) the unique intergenic sequence in the bacteria could be amplified by PCR and then the probe hybridized; or (3) the ligase chain reaction (LCR) can be run using special labeled probes. The hybridization can be detected by a variety of labels including: fluorescence, chemiluminescense, enzymes, antibodies, radioisotopes or a combination of these.

For example, in the PCR assays described herein the conditions in Table 6 were used.

Table 6

PCR Conditions

PRIMERS	STEP		TEMPERATURE	TIME (Min)
Ec1,A113I	Initial	Denature	94°	9
	Cycle	Denature	90°	30
	Cycle	Anneal	50°-60°	1
	Cycle	Extend	65°-70°	5-8
St2B,A113I	Initial	Denature	94°	9
	Cycle	Denature	90°	30
	Cycle	Anneal	50°-60°	1
	Cycle	Extend	70°	5
BsZ,A113I	Initial	Denature	94°	9
	Cycle	Denature	90°	30
	Cycle	Anneal	60°	1
	Cycle	Extend	70°	5
A112I,A113I	Initial	Denature	94°	9
	Cycle	Denature	90°	30
	Cycle	Anneal	50°-55°	1
	Cycle	Extend	65°	8
A112I,A114I	Initial	Denature	94°	9
	Cycle	Denature	90°	30
	Cycle	Anneal	55°	1
	Cycle	Extend	70°	5

One skilled in the art readily appreciates that hybridization conditions are dependent on salt concentration

and temperatures and readily knows how to adjust these to adjust the hybridization sensitivity.

Example 7

MMS Operon Unique Intergenic Operon Identification by Use of Fluorescent Probes

To directly identify a species of specific bacteria on a microscope slide the species specific intergenic sequences of the MMS operon was used. An oligonucleotide was synthesized complementary to the MMS operon intergenic sequence. This oligonucleotide is labeled with fluorescein on its 5′ end by standard procedures. These fluorescent probes were placed on a microscope slide, the bacteria was fixed, the slide washed and the sample visualized by fluorescence microscopy. When the bacteria which contained the unique intergenic sequence was present fluorescence was seen.

Example 8

Ligase Chain Reaction (LCR)

Another method of identification is the LCR method. In this method the 5′ end of a probe is labeled with fluorescein and the 3′ end is labeled with biotin. The unique intergenic sequence probe is split into two segments. The segments, one containing the fluorescein and one containing the biotin label, are added to the bacteria and LCR is run. The bacteria are identified after separation. Multiple bacteria can be simultaneously identified with this procedure if each species specific probe has a different fluorescent label.

Example 9

MMS Operon Intergenic Regions can be Utilized to Recognize All Strains Within an Individual Species

Figure 15 shows genomic DNA from 21 different strains of pathogenic ***E. coli*** isolated as specimens grown from bodily fluids of patients (cerebrospinal fluid, blood, urine, etc.). These specimens were used in a reaction with the ***E. coli*** species specific intergenic sequence primer MMS Ecl. As in Figure 14, MMS ALL3I was used as the other primer in the standard PCR reaction. All the ***E. coli*** strains amplify the expected size fragment. The negative control, genomic DNA from ***S. typhimurium***, did not amplify.

Example 10

Specifics Specific Hybridization in ***S. typhimurium***

In Figure 16, genomic DNA from 22 different strains of ***S. typhimurium*** were isolated from patient bodily fluids and were utilized. Note amplification of the expected size DNA fragment in all ***S. typhimurium*** strains when the species specific intergenic sequence primer was used.

Example 11

Targeting Unique Intergenic Sequences from the MMS Operon to Detect Specific bacterial Species

The unique intergenic sequences from the macromolecular synthesis operon which can be obtained by a variety of procedures, including the novel methods described herein, can be used as targets for a DNA based probe diagnostic test to demonstrate the presence of a specific bacterial species in: (1) a clinical specimen (cerebrospinal fluid, urine, blood, etc.); (2) a food sample (chicken, oysters, infant formula, etc.); or (3) a water sample (ocean water, sewage system, city water supply, etc.). The presence of any bacteria is determined by virtue of the presence of homologous sequences from the MMS operon. The DNA from a particular bacterial species is determined by the presence of a unique intergenic species specific sequence from the macromolecular synthesis operon.

Example 12

Use of Conserved Homologous Regions from the MMS Operon to Detect the Presence of Bacteria

Since the macromolecular synthesis operon is found in all bacterial species, and the expressed sequences or genes have conserved homologous regions, oligonucleotide probes can be designed from a consensus of the conserved homologous regions of several different bacterial species to enable the identification of any bacterial species. To make the consensus sequence all of the non-conserved bases are replaced with an inosene (I). Inosine will base pair by hydrogen bonding with any of the four usual bases A, C, G or T. Alternatively, multiple oligonucleotides can be synthesized with different bases at the non-conserved locations to yield a mixture of degenerate oligonucleotides. The degeneracy can be complete, placing all four possible bases (N=A, T, G and C) at a specific location or partial; placing less than four bases, based on deductions by examination of base sequences at the non-conserved position from a number of bacterial species. A mixture of the oligonucleotides can then be used to detect the presence of any bacteria. (Figure 18). This is true because all bacteria have a macromolecular synthesis operon and all bacteria have conserved homologous regions within the expressed genes of the macromolecular synthesis operon. A probe that detects these conserved homologous regions therefore enables the detection of the presence of any bacteria.

Example 13

Use of the Homologous Probe to Make a Clinical Diagnosis of Bacterial Meningitis

Since it is very important to the physicians to be able to distinguish bacterial meningitis from viral meningitis, the homologous probe technique provides a very useful diagnostic test. This diagnostic test is based on the ability of the homologous probe to detect the presence of bacteria in a cerebrospinal fluid (CSF) specimen obtained after lumbar puncture. Normally, CSF fluid is sterile and thus does not contain any virus or bacteria. If bacteria are present in the CSF, the patient, by definition, has bacterial meningitis. Although this is life threatening, specific antibiotic treatment is available. Until the present invention, the standard procedure was to culture the CSF and wait 72 hours to see if any bacterial species grow.

The present invention uses either a consensus homologous sequence or a spinal fluid panel to test for bacterial meningitis. Since this test is quite efficient, quick and accurate, it is no longer necessary to wait 72 hours for bacteria to grow. The CSF is tested for bacteria by determining the presence of bacterial DNA. If bacterial DNA is present, then bacteria is present and thus, the patient has bacterial meningitis. The test can include a consensus sequence probe or a mixture of oligonucleotide probes to the conserved homologous regions from the expressed sequences of the macromolecular synthesis operon. The probes are used to detect the presence of any bacteria. An alternative embodiment of this invention is to use unique intergenic probes from the macromolecular synthesis operon. This allows the further identification of the bacteria. The unique intergenic probes can be used by themselves or in combination with the homologous probes to identify the bacteria. In one test panel the most commonly occurring bacterial pathogens for bacterial meningitis in the neonatal and pediatric age group, ***H. influenza, S. pneumoniae, N. meningitidus***, grpB ***Streptococcus, L. monocytogenes and E. coli***, are used to identify the specific bacterial. species which is present in the CSF.

Figures 19 and 20 show the ***rpsU-dnaG*** and ***dnaG-rpoD*** amplifications for organisms in the spinal fluid panel.

Example 14

A Sexually Transmitted Disease (STD) Panel to Detect the Presence of Bacteria Associated with Sexually Transmitted Disease

In the STD panel probes are made to the Unique Intergenic Region of bacteria associated with sexually transmitted disease. The initial bacteria used in the panel are: ***T. Pallidum*** (the causative agent of syphylis), ***N. gonnorhea*** (the cause of gonnorhea) and ***Chlamydia*** species. The unique intergenic region from each of these bacteria are determined as outlined above for ***L. monocytogenes*** and ***H. influenzae***.

Again, as in the case for the spinal fluid panel, the test includes oligonucleotide probes to the conserved homologous regions from the expressed sequences of the macromolecular synthesis operon to detect the presence of any bacteria. The probes for the MMS operon unique intergenic regions from ***T. pallidum, N. gonnorhea*** and ***Chlamydia*** species are then used to test for the presence of these organisms.

Example 15

Species Specificity of MMS Operon Intergenic Regions

Figure 14 demonstrates species specificity of the MMS operon intergenic sequences. The species specific intergenic sequences MMS Ec1, MMS St2B, MMS Bs2 were utilized as primers in a PCR reaction with the rpoD gene homologous region probe MMS ALL3I used as the other primer. Standard reaction conditions were utilized for polymerase chain reaction (PCR). When MMS Ec1 is used only ***E. coli*** genomic DNA samples amplify the expected size fragment. The genomic DNA from ***S. typhimurium*** and ***B. subtilis*** does not amplify. Nor do yeast (***S. cerevisiae***) or human DNA (***H. sapiens***) negative controls.

It is further shown in Figure 14 that when MMS St2B is used, only ***S. typhimurium*** genomic DNA amplifies the expected size fragment and when MMS Bs2 is used only ***B. subtilis*** genomic DNA amplifies the expected size DNA fragment. When homologous region probes are used as primers, MMS ALL1I plus MMS ALL3I, or MMS ALL2I plus MMS ALL4I, all three species, ***E. coli, S. typhimurium*** and ***B. subtilis***, amplify the expected size fragment. Negative controls include human genomic DNA and yeast, ***Sacharomyces cervisae*** genomic DNA.

Example 16

PCR Amplification of Conserved Homologous Regions

The MMS operon structure, and regions within expressed sequences or genes in the MMS operon, are conserved in all bacteria.

Oligonucleotide probes to homologous regions within the ***dnaG*** gene (MMS ALL1I) and the ***rpoD*** gene (MMS ALL3I) were used as primers in a PCR reactions with genomic DNA from various Eubacteria. PCR amplification of a specific size fragment will only take place if both (i) the homologous region probes are conserved in the ***dnaG*** and ***rpoD*** genes from these organisms and (ii) that the genes are contiguous or adjacent (thus confirming the MMS operon structure). In Figure 17, every bacteria tested amplified a specific single DNA fragment. The different sizes of some species indicates that the homoloaous region of ***dnaG*** and ***rpoD*** genes are located at different distances apart. Thus, even though the sequence length was not conserved, the sequences still contained the homologous sequence. Negative controls, including human, yeast and the use of only one primer (either MMS ALL1I or MMS ALL-3I), and a reaction where no genomic DNA is placed in the reaction demonstrate no amplification.

These results demonstrate that: (1) the amplified DNA fragment must contain the unique intergenic region between ***dnaG*** and ***rpoD*** from these various microorganisms; (2) that the unique intergenic sequence be isolated and determined using the procedure of the present invention; (3) the intergenic regions of the MMS operon are species specific; (4) the MMS operon intergenic regions can be utilized to recognize all strains within an individual species (this is contrary to present day immunological methods which recognize surface antigens on a cell and do not recognize all strains within a species); (5) expressed sequences (genes) within the MMS operon are conserved in all bacteria and regions of homology within the ***dnaG*** gene and ***rpoD*** gene can be used to identify the presence of any bacteria by identifying these homologous regions within ***dnaG*** and ***rpoD***; and (6) the macromolecular synthesis operon structure is conserved in all bacteria.

One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as, those inherent therein. The oligonucleotides, antibiotics, compounds, methods, procedures and techniques described herein are presently representative of preferred embodiments, are intended to be exemplary, and are not intended as limitations on the scope. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention or defined by the scope of the appended claims.

Claims

1. A method for treating bacterial infections comprising the step of interrupting the expression of a macromolecular synthesis operon by hybridizing an antisense oligonucleotide antibiotic to a mRNA transcribed from said macromolecular synthesis operon wherein the antisense oligonucleotide antibiotic is selected from the group consisting of

5′ CAITGCTTTGGITGIGGIGCGIIIGGCAA 3′
5′ TTGCCIIICGCICCICAICCAAAGCAITG 3′
5′ CANTGCTTTGGNTGNGGNGCGNNNGGCAA 3′
5′ TTGCCNNNCGCNCCNCANCCAAAGCANTG 3′
5′ ACITAIGCIACITGGTGGATGIGICAGGC 3′
5′ ACNTANGCNACNTGGTGGATCNGNCAGGC 3′
5′ GCCTGICIGATCCACCAIGTIGCITAIGT 3′
5′ GCCTGNCNGATCCACCANGTNGCNTANGT 3′
5′ TTIGCTTCGATITGICGIATACG 3′,
5′ TTNGCTTCGATNTGNCGNATACG 3′
5′ ACGAGCCGTTCGACGTAGCTCTGCG 3′
5′ CGGCGTGCGTTTTCGCGAGCCAGT 3′ and
5′ ACATGCCGGTAATTAAAGTACGTG 3′.

2. A method of claim 1, wherein the antisense oligonucleotide antibiotic hybridizes to a bacterial homologous sequence in the mRNA transcribed from said macromolecular synthesis operon.

3. A method of claim 1, wherein the antisense oligonucleotide antibiotic binds to an intergenic sequence, said intergenic sequence is unique for each species of bacteria.

4. A method of claim 3, wherein the antisense oligonucleotide antibiotic is selected from the group consisting of:

5′ TTATCGTTGGCGGTAAACAACCGTTGG 3′;

which antibiotic hybridizes to the transcribed mRNA of E. coli.

5. A method of claim 3, wherein the antisense oligonucleotide antibiotic is selected from the group consisting of:

5′ CGGCGCTTACGCAAGTCAGCGACA 3′ and
5′ CGACAGCTATACCGTCGACACC 3′;

which antibiotic hybridizes to the transcribed mRNA of *S. typhimurium*.

6. A method of claim 3, wherein the antisense oligonucleotide antibiotic is selected from the group consisting of:

5′ GGGATTTGCACTAAAGCATCG 3′ and
5′ GATCGCTTAACCTCATCATG 3′;

which antibiotic hybridizes to the transcribed mRNA of *B. subtilis*.

7. A method of claim 3, wherein the antisense oligonucleotide antibiotic is selected from the group consisting of:

5′ CCACGCGGATTGGGCGTAACGCTCTTGGG 3′ and
5′ CCCAAGAGCGTTACGCCCAATCCGCGTGG 3′;

which antibiotic hybridizes to the transcribed mRNA of *S. coelicolor.*

8. A method of claim 3, wherein the antisense oligonucleotide antibiotic is:

5′ GTCGGTGTAGGAAGTTTTTCTAGGGCCG 3′;

which antibiotic hybridizes to the transcribed mRNA of *C. trachomatis.*

9. A method of claim 3, wherein the antisense oligonucleotide antibiotic is:

5′ CGTGTCATGCTCGAAATCGTCCAACTC 3′;

which antibiotic hybridizes to the transcribed mRNA of *L. monocytogenes.*

10. A method of claim 3, wherein the antisense oligonucleotide antibiotic is:

5′ CGCCCATGCAACCGGTTTGAGTTCGCG 3′;

which antibiotic hybridizes to the transcribed mRNA of *M. xanthus.*

11. A method of identifying bacteria, comprising the steps of:

hybridizing a unique intergenic antisense oligonucleotide of about 10 to 29 mer to a mRNA transcribed from a macromolecular synthesis operon; and

measuring the amount of said hybridization; wherein each unique intergenic antisense oligonucleotide only binds to a specific bacteria wherein the oligonucleotide is selected from the group consisting of:

5′ TTATCGTTGGCGGTAAACAACCGTTGG 3′;

and the bacteria is identified as *E. coli.*

12. A method of claim 11, wherein the oligonucleotide is selected from the group consisting of:

5' CGGCGCTTACGCAAGTCAGCGACA 3'

and

5' CGACAGCTATACCGTCGACACC 3';

and the bacteria is identified as ***S. typhimurium.***

13. A method of claim 11, wherein the oligonucleotide is selected from the group consisting of:

5' GGGATTTGCACTAAAGCATCG 3'

and

5' GATCGCTTAACCTCATCATG 3';

and the bacteria is identified as ***B. subtilis.***

14. A method of claim 11, wherein the oligonucleotide is

5' GTCGGTGTAGGAAGTTTTTCTAGGGCCG 3';

and the bacteria is identified as ***C. trachomatis.***

15. A method of claim 11, wherein the oligonucleotide is selected from the group consisting of

5' CCACGCGGATTGGGCGTAACGCTCTTGGG 3'

and

5' CCCAAGAGCGTTACGCCCAATCCGCGTGG 3';

and the bacteria is identified as ***S. coelicolor.***

16. A method of claim 11, wherein the oligonucleotide is

5' CGTGTCATGCTCGAAATCGTCCAACTC 3';

and the bacteria is identified as ***L. monocytogenes.***

17. A method of claim 11, wherein the oligonucleotide is

5' CGCCCATGCAACCGGTTTGAGTTCGCG 3';

and the bacteria is identified as ***M. xanthus.***

18. A method of identifying the presence or absence of bacteria comprising the steps of:

hybridizing a homologous antisense oligonucleotide of about 10 to 29 mer to a mRNA transcribed from a macromolecular synthesis operon; and

measuring the amount of said hybridization, wherein said homologous antisense oligonucleotide hybridized to all bacteria containing said sequence.

19. A method of claim 18, wherein the sequence is selected from the group consisting of

5' CAITGCTTTGGITGIGGIGCGIIIGGCAA 3',
5' TTGCCIIICGCICCICAICCAAAGCAITG 3',
5' CANTGCTTTGGNTGNGGNGCGNNNGGCAA 3',
5' TTGCCNNNCGCNCCNCANCCAAAGCANTG 3',
5' ACITAIGCIACITGGTGGATGIGICAGGC 3',
5' ACNTANGCNACNTGGTGGATCNGNCAGGC 3',
5' GCCTGICIGATCCACCAIGTIGCITAIGT 3',
5' GCCTGNCNGATCCACCANGTNGCNTANGT 3',
5' TTIGCTTCGATITGICGIATACG 3',
5' TTNGCTTCGATNTGNCGNATACG 3',
5' ACGAGCCGTTCGACGTAGCTCTGCG 3',
5' CGGCGTGCGTTTTCGCGAGCCAGT 3'and
5' ACATGCCGGTAATTAAAGTACGTG 3'.

20. An antibiotic, comprising:

at least a 10 mer oligonucleotide, wherein said oligonucleotide is complementary to a sense strand of a macromolecular synthesis operon and binds to a mRNA transcribed by said sense strand wherein said
oligonucleotide is selected from the group consisting of:

5' CAITGCTTTGGITGIGGIGCGIIIGGCAA 3',
5' TTGCCIIICGCICCICAICCAAAGCAITG 3',
5' CANTGCTTTGGNTGNGGNGCGNNNGGCAA 3',
5' TTGCCNNNCGCNCCNCANCCAAAGCANTG 3',
5' ACITAIGCIACITGGTGGATGIGICAGGC 3',
5' ACNTANGCNACNTGGTGGATCNGNCAGGC 3',
5' GCCTGICIGATCCACCAIGTIGCITAIGT 3',
5' GCCTGNCNGATCCACCANGTNGCNTANGT 3',

5' TTIGCTTCGATITGICGIATACG 3',
5' TTNGCTTCGATNTGNCGNATACG 3',
5' ACGAGCCGTTCGACGTAGCTCTGCG 3',
5' CGGCGTGCGTTTTCGCGAGCCAGT 3',
5' ACATGCCGGTAATTAAAGTACGTG 3',
5' GGGATTTGCACTAAAGCATCG 3', 5' GATCGCTTAACCTCATCATG 3',
5' GTCGGTGTAGGAAGTTTTTCTAGGGCCG 3',
5' TTATCGTTGGCGGTAAACAACCGTTGG 3',
5' CCACGCGGATTGGGCGTAACGCTCTTGGG 3',
5' CCCAAGAGCGTTACGCCCAATCCGCGTGG 3',
5' CGTGTCATGCTCGAAATCGTCCAACTC 3',
5' CGCCCATGCAACCGGTTTGAGTTCGCG 3',
5' CGGCGCTTACGCAAGTCAGCGACA 3', and
5' CGACAGCTATACCGTCGACACC 3'.

21. An antibiotic of claim 20, further comprising:
a carrier molecule linked to said oligonucleotide, wherein said carrier molecule regulates the uptake of said oligonucleotide into the bacterium.

22. An antibiotic of claim 21, wherein the carrier molecule is an amino acid.

23. An antibiotic of claim 20, wherein said oligonucleotide is derivatized at the 3′ or 5′ termini to prevent degradation of said oligonucleotide.

24. An antibiotic of claim 23 wherein a phosphorothioate linked nucleotide is added to the 3′ termini by derivatization.

25. An antibiotic of claim 23, wherein a primary amine is added to either the 3′ or 5′ termini.

26. A method of identifying bacteria, comprising the steps of:
treating a macromolecular synthesis operon to form single stranded DNA;
hybridizing an antisense oligonucleotide of about 10 to 29 mer to a unique intergenic sequence in the single stranded DNA of the macromolecular synthesis operon; and
measuring the amount of said hybridization wherein the oligonucleotide is selected from the group consisting of

5′ TTATCGTTGGCGGTAAACAACCGTTGG 3′;

and the bacteria is identified as *E. coli.*

27. A method of claim 26, wherein the oligonucleotide is selected from the group consisting of

5' CGGCGCTTACGCAAGTCAGCGACA 3'

and

5' CGACAGCTATACCGTCGACACC 3';

and the bacteria is identified as *S. typhimurium.*

28. A method of claim 26,wherein the oligonucleotide is selected from the group consisting of

5' GGGATTTGCACTAAAGCATCG 3'

and

5' GATCGCTTAACCTCATCATG 3';

and the bacteria is identified as ***B. subtilis.***

29. A method of claim 26, wherein the oligonucleotide is

5' GTCGGTGTAGGAAGTTTTTCTAGGGCCG 3';

and the bacteria is identified as ***C. trachomatis.***

30. A method of claim 26, wherein the oligonucleotide is selected from the group consisting of

5' CCACGCGGATTGGGCGTAACGCTCTTGGG 3'

and

5' CCCAAGAGCGTTACGCCCAATCCGCGTGG 3';

and the bacteria is identified as ***S. coelicolor.***

31. A method of claim 26, wherein the oligonucleotide is

5' CGTGTCATGCTCGAAATCGTCCAACTC 3';

and the bacteria is identified as ***L. monocytogenes.***

32. A method of claim 26, wherein the oligonucleotide is

5' CGCCCATGCAACCGGTTTGAGTTCGCG 3';

and the bacteria is identified as ***M. xanthus.***

33. A macromolecular synthesis operon DNA sequence of the formula:

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GCAACTTCTT GGTGCAACAT CGTTTATCAT GATAATTACA          40
AAGCGCTTTA TACCTATCTA ATTGGTTATT TCTGGCAGAA          80
GGTAATGATG CAGATCCAAC GGAAATTTAT GGATAGTGTT         120
CCTGATGCTA CAATGAAAGG ACTTATCAGT AGCCTCGAAA         160
TGGTTATTAG TCCAGATGAA CAAGGTAAAA CACAGTTTGA         200
AGACTATATT AGAAGTCTAA AGCGGTTTAA ATTAGAACAA         240
AAGAAAAAAG AACTTGAGCA AGAGCTAAGC AACTTTAAAT         280
CGTGAAAATG ACAAAGATAA CGAAATTCGT GTCATGCTCG         320
AAATCGTCCA ACTCAACCGT CAGTTAAACA GCGGCCAATT         360
GGATTAATAA CGTTTTAAAA CCGCTAAATG ATGGTATTAT         400
TACCTAAGAG AAGCCTTTTA ATAAGGTTAG CGGCATTTTG         440
GAAGGAGGAA TACAGGCAGT TATGAGTGAT AAAACAAAAA         480
ACACAAAACC AGTTGCTGAA CTAAGTGTTG AGCAAGTAAA         520

AGAAGCCCTG ATAGAAGAAG GTAAGAAAAA GGGGATTTTA         560
ACTTATGCAA AAATCGCTGC CAGATTAGCT CCATTCACTT         600
TGGATTCCGA TCAAATGGAT GAGTATTTAG AACATGTTGG         640
TGAAGCAGGA ATTGAAGTTT CTGACGATGC AGATGATGAG         680
GATCCGGATG AAACAGAACT TGTAAAAGAA GAAACCGAAT         720
CCTTTGATTT AACAGATATG AGTGTACCAC CAGGCGTAAA         760
AATTAATGAC CCTGTTCGCA TGTATCTGAA AGAAATTGGT         800
CGAGTAGACT TACTTACAGC GGATGAAGAA ATTGCCTTAG         840
CAAAACGTAT CGAAGCTGGC GACATTGAAG CCAAAGGACG         880
TCTTGCAGAA GCCAACCTGC GCCTTGTTGT AAGTATTGCA         920
AAACGTTATG TTGGTCGCGG TATGTTATTC CTTGATTTAA         960
TTCAAGAAGG TAACATGGGA CTAATGAAAG CCGTTGAGAA        1000
ATTCGACTTC AATAAAGGAT TTAAATTCAG TACCTATGCA        1040
ACG                                               1043
```

and fragments and derivatives thereof, said fragments and derivatives forming homologous sequences or unique intergenic sequences.

34. A DNA sequence of claim 33 for the ***L. monocytogenes dnaG*** gene, comprising the bases numbered 1 to 282, and fragments and derivatives thereof, said fragments and derivatives forming homologous sequences.

35. A DNA sequence of claim 33 for the ***L. monocytogenes dnaG-rpoD*** intergenic sequence, comprising the bases numbered 283 to 461 and fragments and derivatives thereof, said fragments and derivatives forming a unique intergenic sequence.

36. A DNA sequence of claim 33 for the ***L. monocytogenes rpoD*** gene, comprising the bases numbered 462 to 1043, and fragments and derivatives thereof, said fragments and derivatives forming homologous sequences.

37. A DNA sequence of the formula:

```
AAGCTTAACG GAAGAACATG CAGATTTAAT TAAACGGCTT          40

ACTAACCGGG CGATTATTTG TTATGACGGT GACAGAGCCG          80
GAATTGAAGC AGCCTATAAG GCGGGCACGC TTCTAGTTGA         120
ACGGAATCGT TTAGATGTTT TTGTTTTGCA ACTTCCAGCT         160
GGAAAAGATC CCGATGACTT TATTCGAGCA AGTGGTCCAG         200
AAAAATTCAA AGAAGTTTAT AAGCAACAAC GATCGACTTG         240
GACAGCTTTT AAATTCATTA TTTACGTAGA GAACGTA            277
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and fragments and derivatives thereof, said fragments and derivatives forming homologous sequences.

38. A macromolecular synthesis operon DNA sequence of the formula:

```
ATGCCGGTAA TTAAAGTACG TCAAAACGAA TCATTTGACG          40
TAGCTTTACG TCGTTTCAAA CGCTCTTGCG AAAAAGCGGG          80
AATCTTAGCT GAAATACGCG CTCGCGAATT TTACGAAAAA         120
CCAACTACAA TTCGTAAACG TGAAAATGCA ACACTTGCAA         160
AACGTCACGC AAAACGCAAC GCTCGCGAAA ACGCGCGCAA         200
TACCCGTTTA TACTAATTTA TAGTATTTTC TGACTCGAGT         240
TAAGACAAAC CGTGAATCCT TTGGACTCAC GGTTTTGTTA         280
CTTTAAGGCA CAACAAAAAT CTACGCCAAA AACGACCGCA         320
CTTTCACACC ACGATCACGG AGGCTCGACA ATGAAAGGTT         360
CTATTCCACG CCCCTTTATT GATGATTTGC TGACAAAGTC         400
CGATATTGTC GATGTGATTA ACACGCGCGT AAAACTAAAA         440
AAAGCTGGCC GCGATTATCA AGCCTGCTGC CCTTTCCATC         480
ACGAAAAAAC ACCATCCTTC ACAGTTAGCC AAAAGAAACA         520
GTTTTATCAC TGCTTTGGCT GCGGCGCG                      548
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and fragments and derivatives thereof, said fragments and derivatives forming homologous sequences or unique intergenic sequences.

39. A DNA sequence of claim 38 for the ***H. influenzae rpsU*** gene, comprising bases numbered 1 to 213, and fragments and derivatives thereof, said fragments and derivatives forming homologous sequences.

40. A DNA sequence of claim 38 for the ***H. influenzae rpsU-dnaG*** intergenic sequence, comprising the bases numbered 214 to 350 and fragments and derivatives thereof, said fragments and derivatives forming a unique intergenic sequence.

41. A DNA sequences of claim 38 for the ***H. influenzae dnaG*** gene, comprising the bases numbered 351 to 548 and fragments and derivatives thereof, said fragments and derivatives forming homologous sequences.

42. A method of isolating macromolecular synthesis operon intergenic sequences comprising the steps of:

performing PCR on a chromosomal DNA isolated from bacteria, wherein primers are selected from the group of primers complementary to homologous sequences in the ***dnaG*** and ***rpoD*** genes, and complementary to homologous sequences in the ***rpsU*** and ***dnaG*** genes;

recovering the amplified product; and

identifying the intergenic sequence in said amplified product by DNA sequence analysis.

43. A method for determining homologous sequences and unique intergenic sequences comprising the steps of:

comparing DNA sequence obtained by the method of claim 42 to all other known macromolecular synthesis operon sequences from various bacterial species;

analyzing dot matrix plots to determine the homology to the expressed sequences ***rpoD, dnaG*** and ***rpdU***; and

identifying the intergenic sequence from said comparison.

44. An assay for detecting the presence of bacteria in a sample selected from the group of clinical specimen, food specimen, and water specimen comprising:

hybridizing a homologous antisense oligonucleotide of about 10 to 29 mer to a mRNA transcribed from a macromolecular synthesis operon or a single stranded bacterial DNA; and

measuring the amount of said hybridization.

45. An assay of claim 44, wherein said bacteria are identified by further hybridizing a unique intergenic antisense oligonucleotide of about 10 to 29 mer to said mRNA or single stranded DNA.

46. An assay of claim 44,for detecting bacterial meningitis, wherein the sample is CSF fluid; and

the unique antisense oligonucleotide includes sequences selected from the macromolecular synthesis operon sequence of ***H. influenzae, S. pneumoniae, N. meningitiders***, group ***B Streptococcus L. monocytogenes*** and ***E. coli.***

47. An assay of claim 44 for detecting bacteria causing sexually transmitted disease, wherein the unique antisense oligonucleotide includes sequences selected from the macromolecular synthesis operon sequences of ***T. pallidum. V. gonnorhea*** and ***Chlamydia*** species.

48. An assay of claim 44 for detecting bacteria causing food poisoning, wherein the unique antisense oligonucleotide includes sequences selected from the macromolecular synthesis operon sequences of ***Lysteria. Vibrio cholera,*** and ***Salmonella.***

49. An assay of claim 44 for detecting bacteria contaminated water, wherein the unique antisense oligonucleotide includes sequences selected from the macromolecular synthesis operon sequence of gram negative enteric bacteria.

50. A kit for detecting or identifying bacteria comprising:

a container having an oliaonucleotide selected from the group consisting of

5' GGCCCCGATTTTTAGCAA 3', 5' CTTGCGTAAGCGCCGGGG 3',

5' TATTCGATGCTTTAGTGC 3',

5' CATCCAAAGCAGTGGTAAAACTGTTT 3',

5' TCACCGATCGGCGTTTCCA 3',

5' CAITGCTTTGGITGIGGIGCGIIIGGCAA 3',
5' TTGCCIIICGCICCICAICCAAAGCAITG 3',
5' CANTGCTTTGGNTGNGGNGCGNNNGGCAA 3',
5' TTGCCNNNCGCNCCNCANCCAAAGCANTG 3',
5' ACITAIGCIACITGGTGGATGIGICAGGC 3',
5' ACNTANGCNACNTGGTGGATCNGNCAGGC 3',
5' GCCTGICIGATCCACCAIGTIGCITAIGT 3',
5' GCCTGNCNGATCCACCANGTNGCNTANGT 3',
5' TTIGCTTCGATITGICGIATACG 3',
5' TTNGCTTCGATNTGNCGNATACG 3',
5' ACGAGCCGTTCGACGTAGCTCTGCG 3',
5' CGGCGTGCGTTTTCGCGAGCCAGT 3'and
5' ACATGCCGGTAATTAAAGTACGTG 3',
5' GGGATTTGCACTAAAGCATCG 3',
5' GATCGCTTAACCTCATCATG 3',
5' GTCGGTGTAGGAAGTTTTTCTAGGGCCG 3',
5' TTATCGTTGGCGGTAAACAACCGTTGG 3',
5' CCACGCGGATTGGGCGTAACGCTCTTGGG 3',
5' CCCAAGAGCGTTACGCCCAATCCGCGTGG 3',
5' CGTGTCATGCTCGAAATCGTCCAACTC 3',
5' CGCCCATGCAACCGGTTTGAGTTCGCG 3',
5' CGGCGCTTACGCAAGTCAGCGACA 3', and
5' CGACAGCTATACCGTCGACACC 3' and combination thereof.

51. A kit of claim 50 further comprising controls.

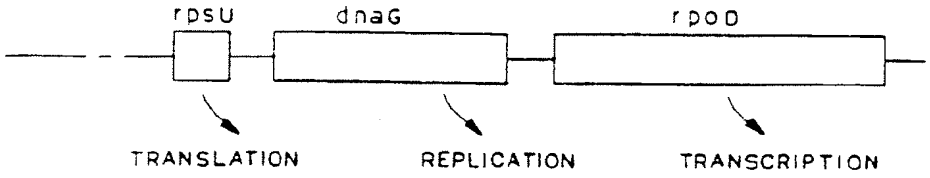


FIG. 1

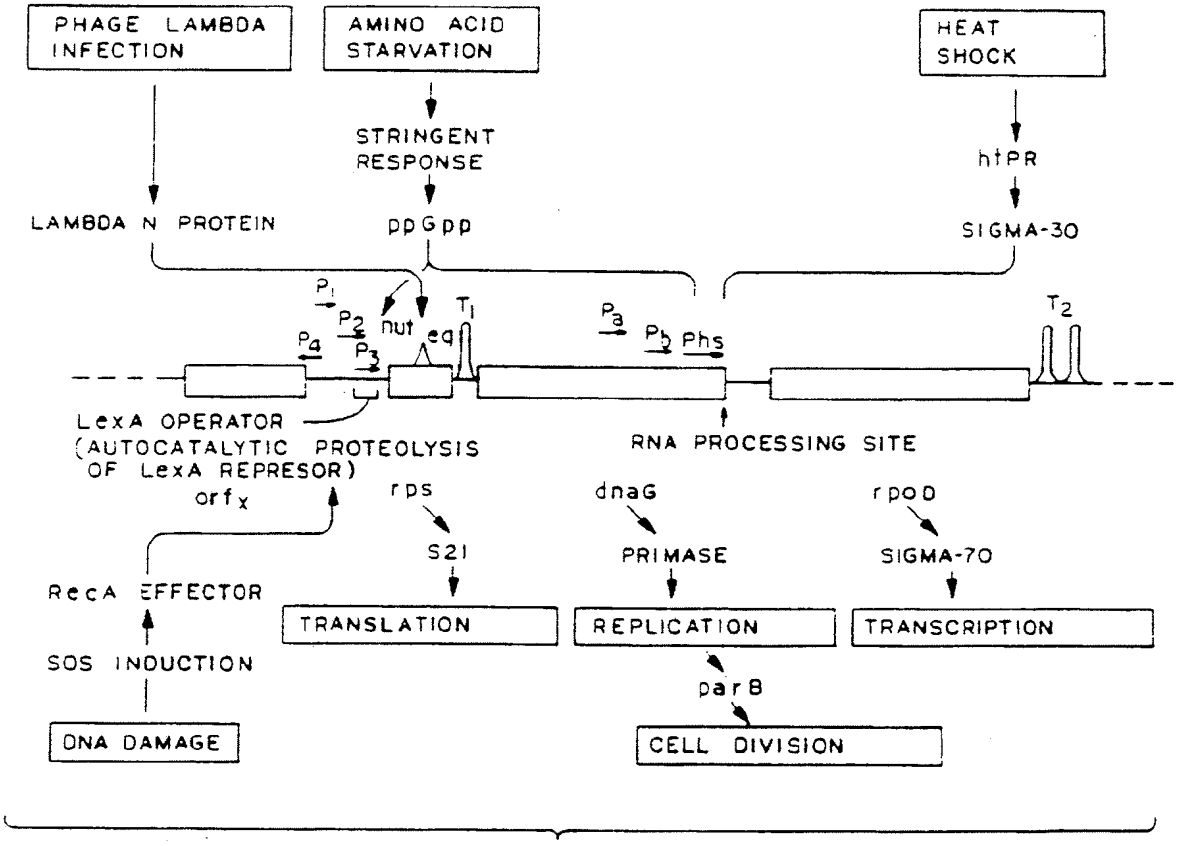


FIG. 2

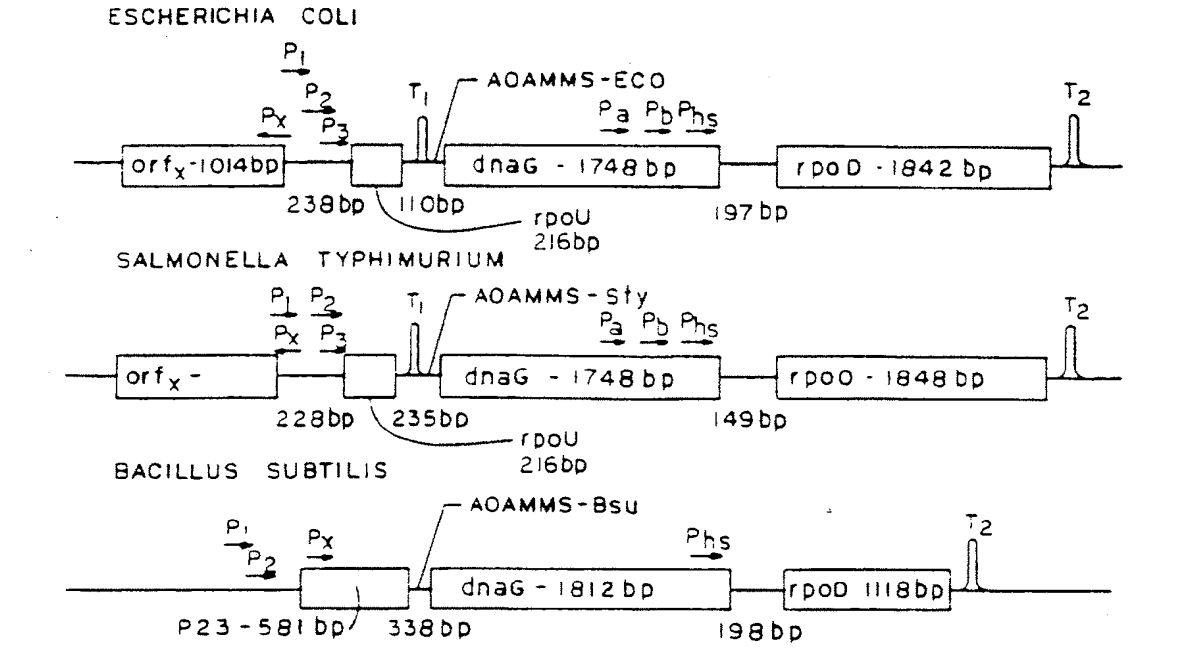


FIG. 3

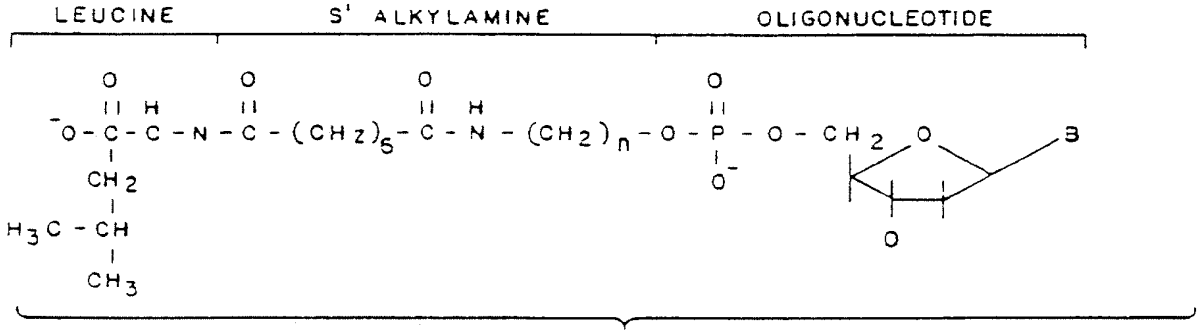
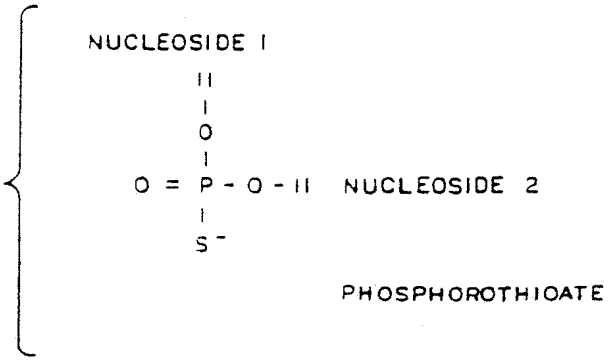


FIG. 4

FIG. 5



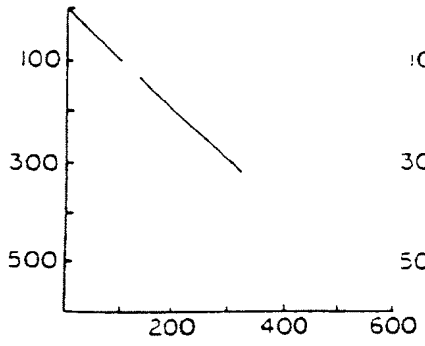


FIG. 6A

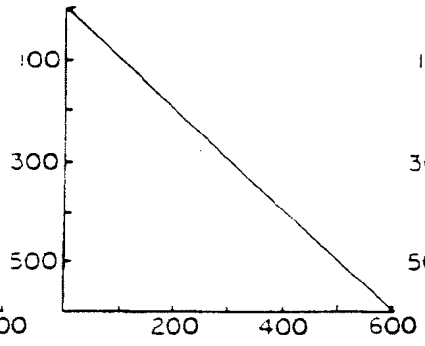


FIG. 6B

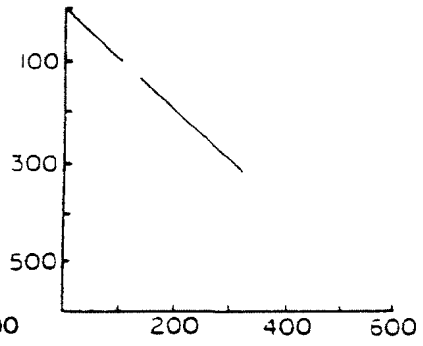


FIG. 6C

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E. COLI          : MAGRIPRVFINDLLARTDIVDLIDARVKLKKQGKNFHACCPFHNEKTPSFTVNGEKQFYH
S. TYPHYMURIUM   : ----------V----------Y----------
B. SUBTILIS      : -GN---DEIVDQVQKSA---EV-GDY-Q-----R YFGL----G S----S-SPD--IF-

E. COLI          : CFGCGAHGNAIDFLMNYDKLEFVETVEELAAMHNLEVPFE.AGSGPSQIERHQRQTLYQL
S. TYPHYMURIUM   : ----------I-Y-.--T-L----------N----
B. SUBTILIS      : ------G--VFS--RQMEGYS-A-S-SII--DKYQIDF-DDITVIISGARP-SSGE-KMAEA

E. COLI          : MDGLNTFYQQSL.QQPVATSARQYLEKRGLSHEVIARFAIGFAPPGWDNVLKRFGGNPEN
S. TYPHYMURIUM   : -N---D------.TH-A-KP--D--Q-----A-I-Q----------A-----N-SD-
B. SUBTILIS      : HEL-KK--HHL-INTKEGQE-LD--LS--FTK-L-NE-Q--Y-LDS--FIT-FLVKRGFS

E. COLI          : RQSLIDAGMLVTNDQGRSY.DRFRERVMFPIRDKRGRVIGFGGRVLGNDTPKYLNSPETD
S TYPHYMURIUM    : KAL-L-----N-E--ST-.----N----------
B. SUBTILIS      : EAQMEK--L-IRRED-SG-I-----N------II-HH-A-VA-S--A--SQQ---M-----P

E. COLI          : IFHKGRQLYGLYEAQQDNAEPNRLLVVEGYMDVVALAQYGINYAVASLGTSTTADHIQLL
S. TYPHYMURIUM   : ----------YS--Q----------D---MHM-
B. SUBTILIS      : L---SKL--NF-K-RLHIRKQE-AVLF--FA--YTAVSSDVKESI-TM---L-D--VKI-

E. COLI          : FRATNNVICCYDGDRAGRDAAWRA
S. TYPHYMURIUM   : ----------
B. SUBTILIS      : R-NVEEI-L---S-K--YE-TLK-
```

FIG 6D

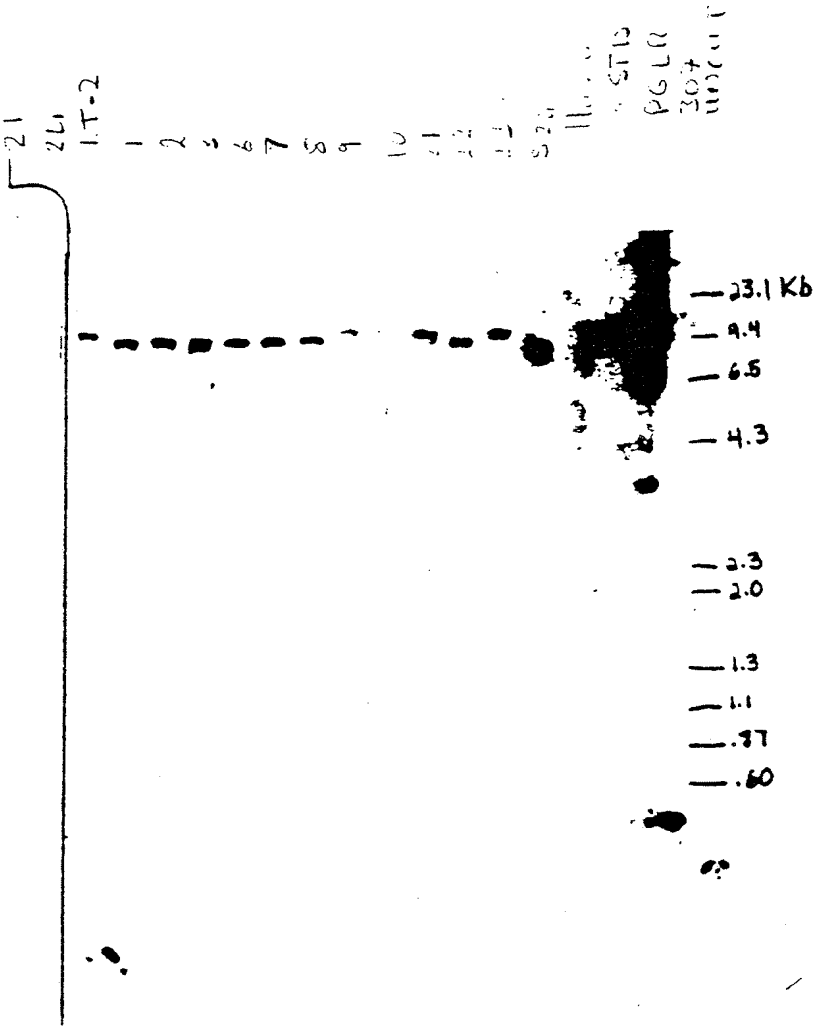

FIG. 7

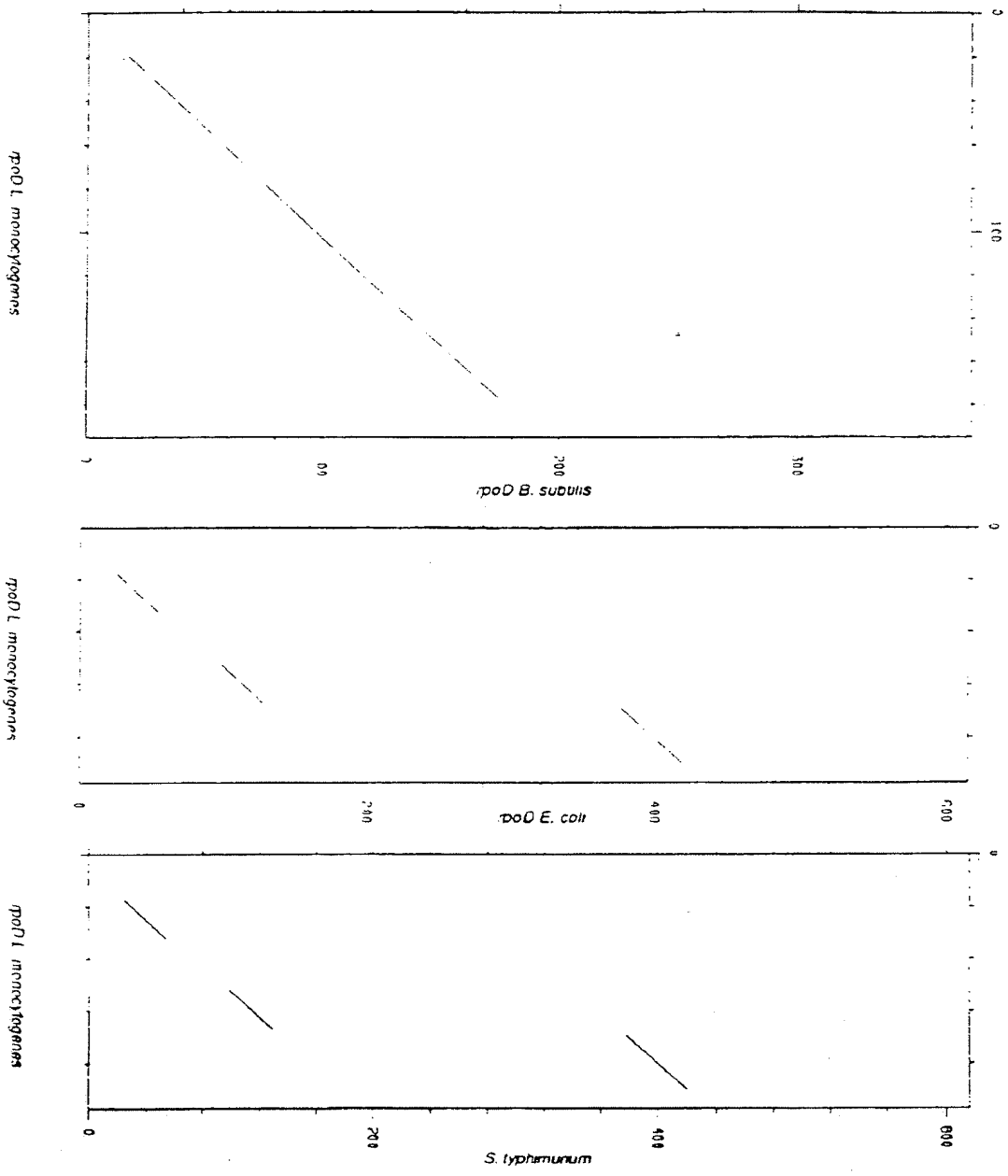

Figure 8

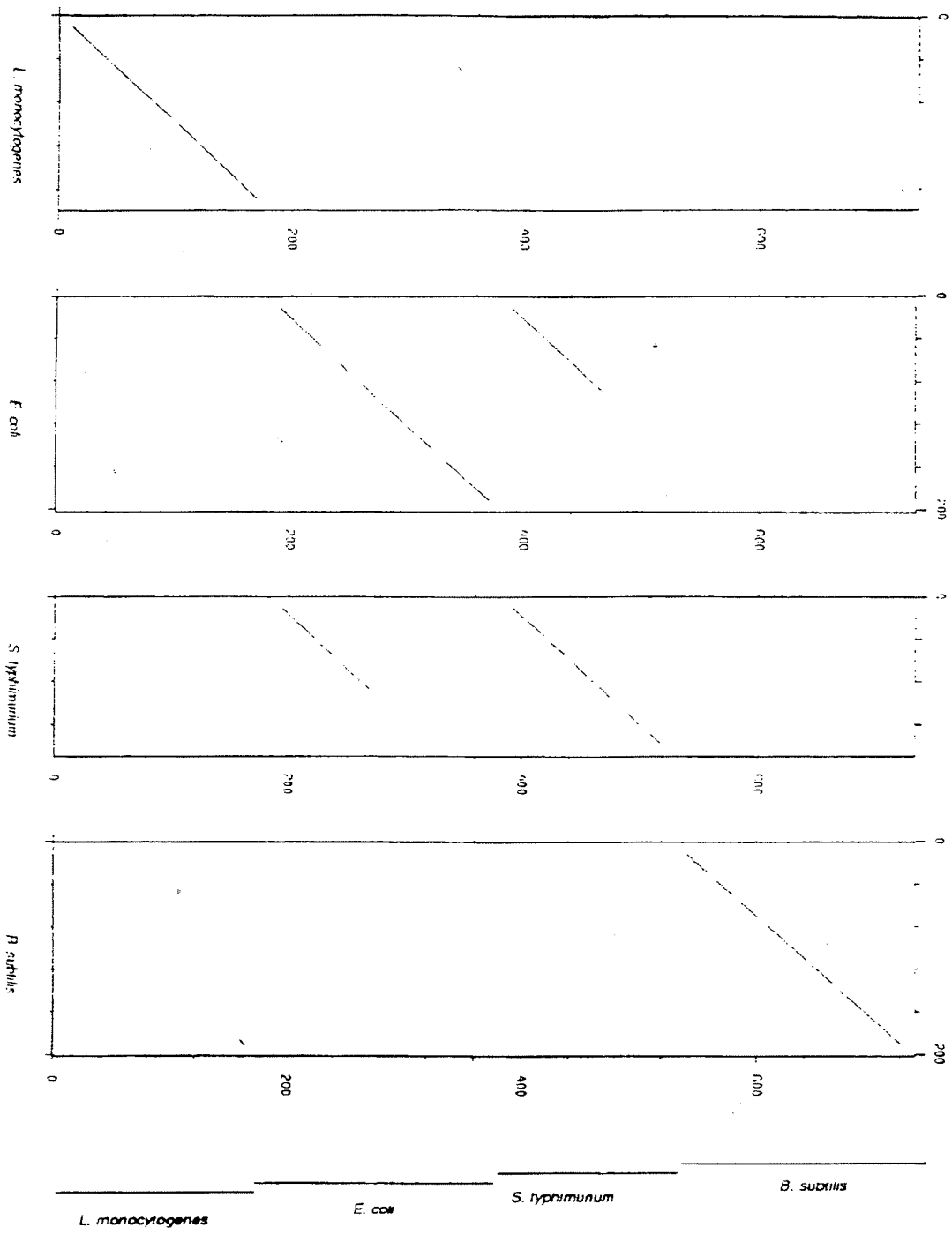

Figure 9

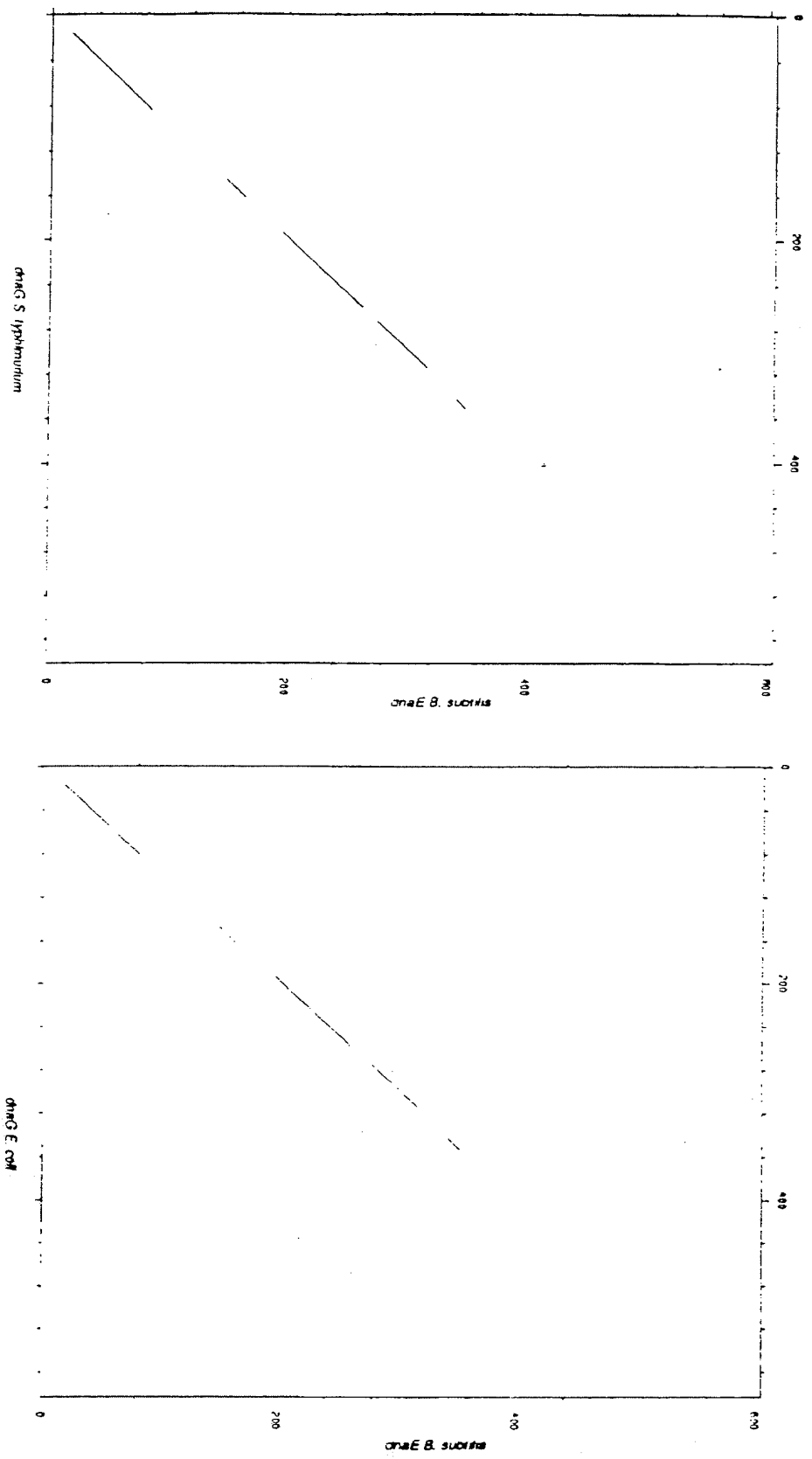

Figure 10

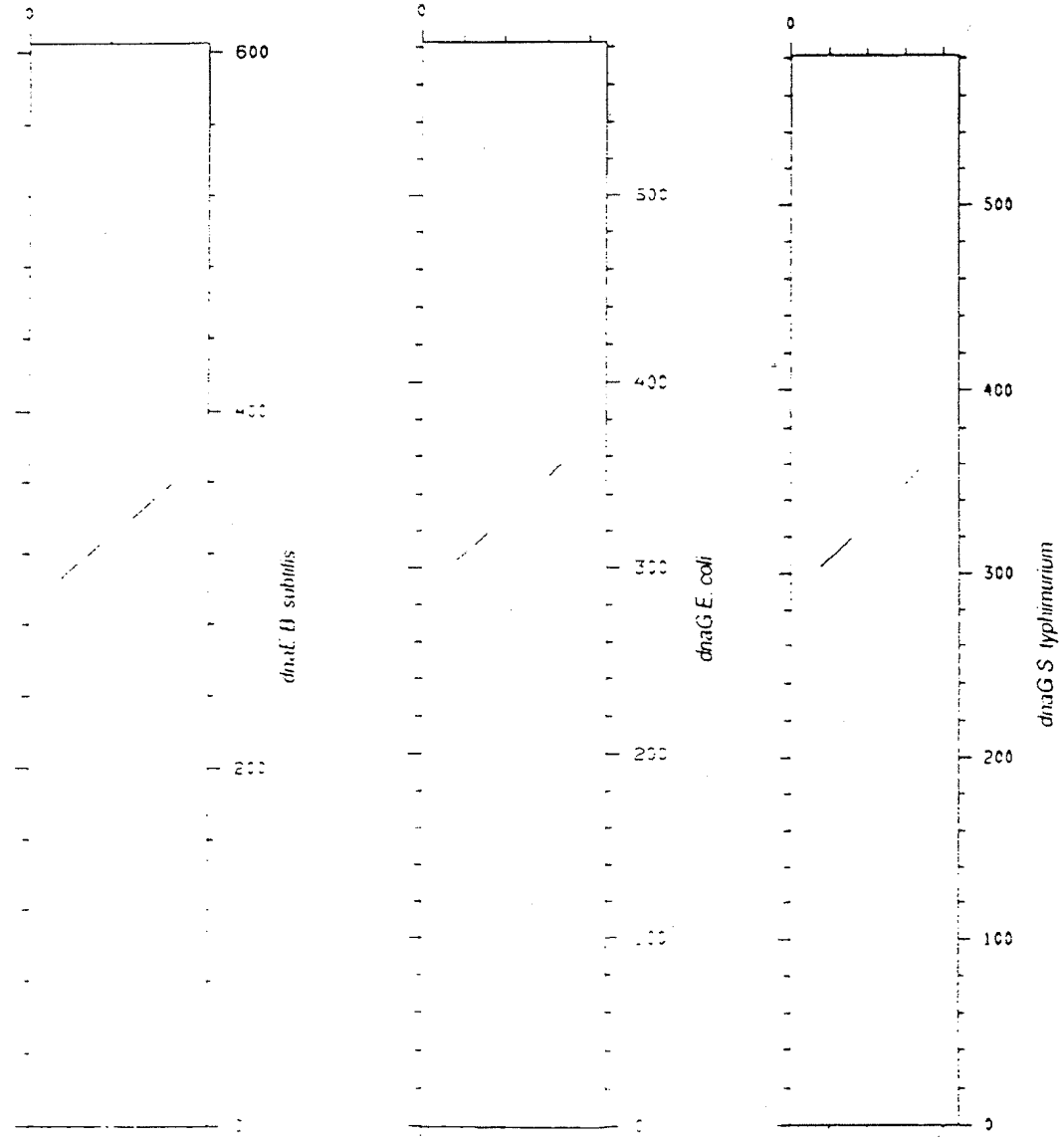

Figure 11

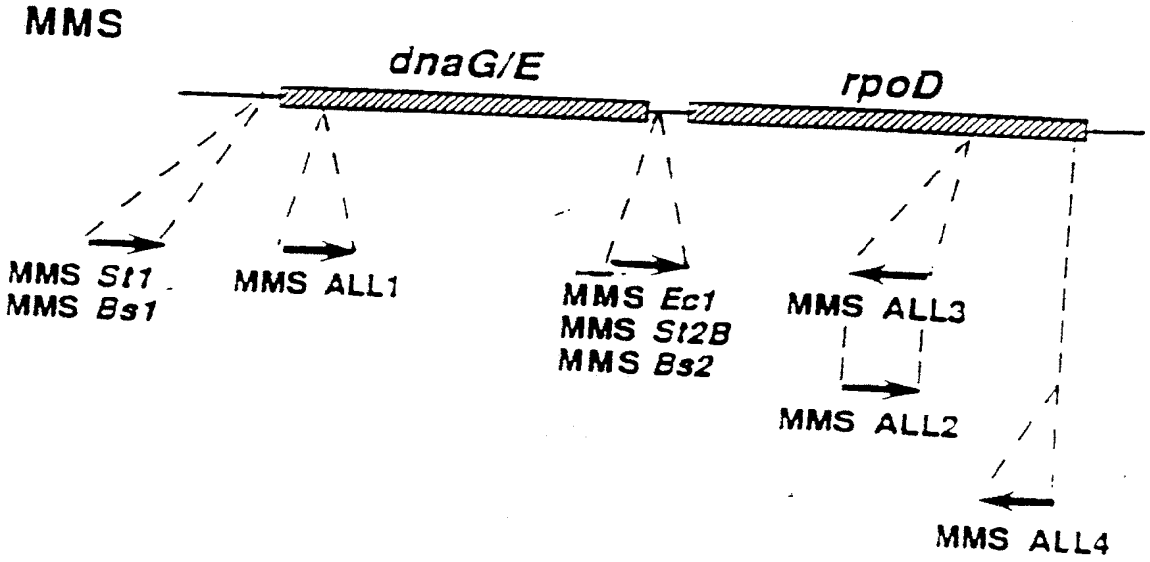

Figure 12

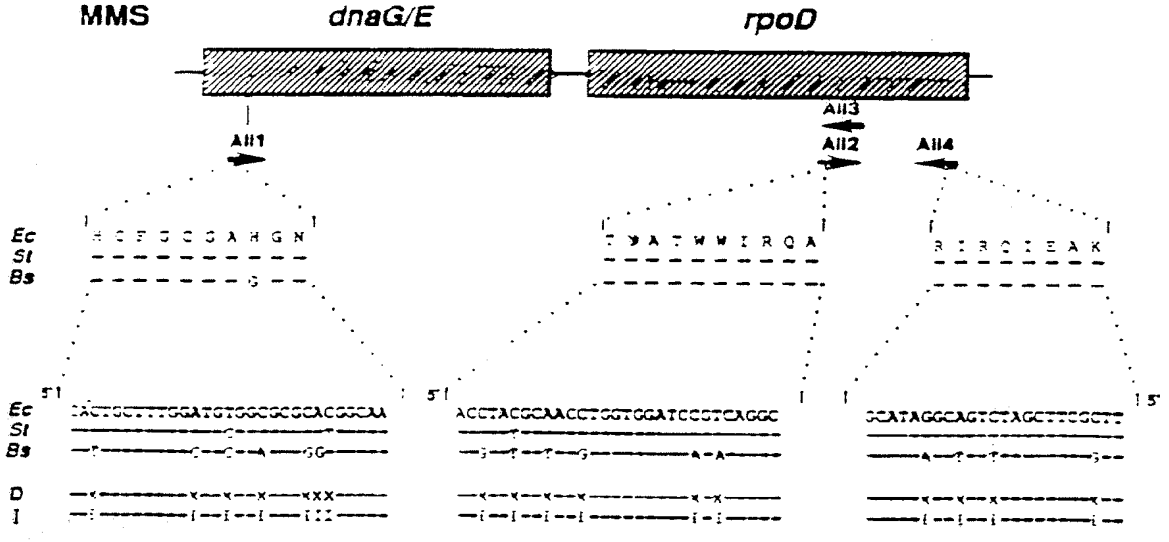

Figure 13

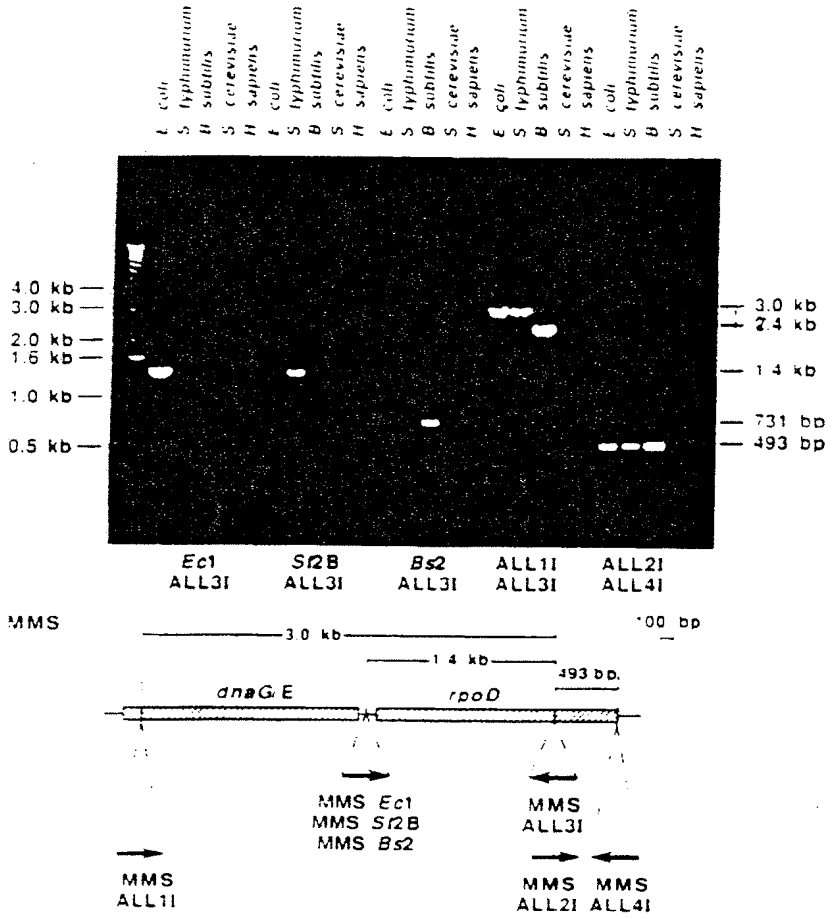

Figure 14

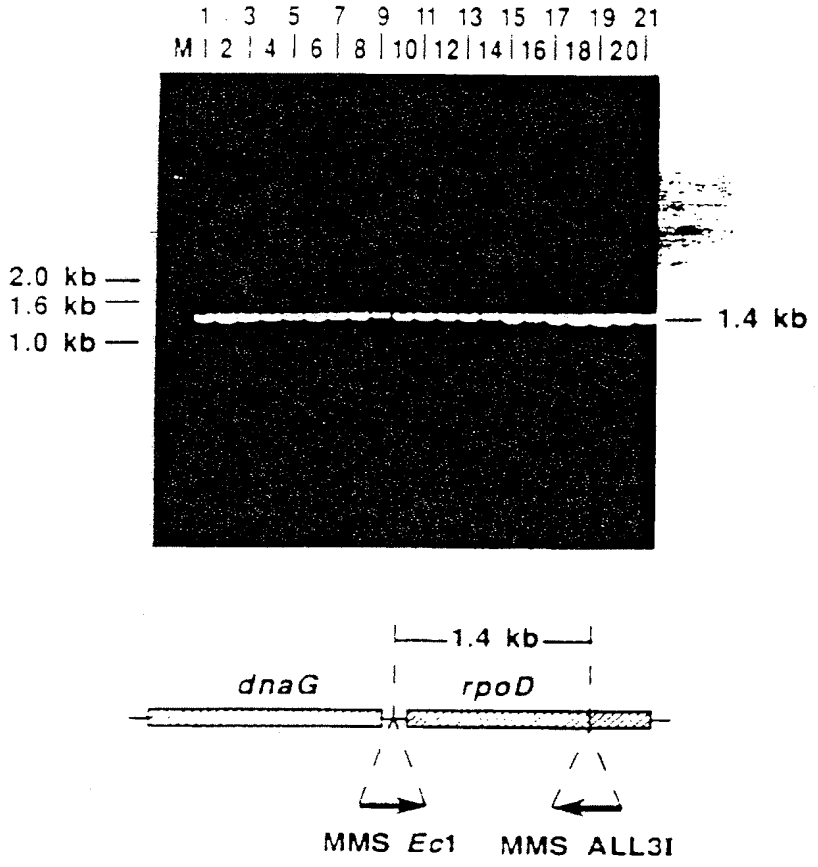

Figure 15

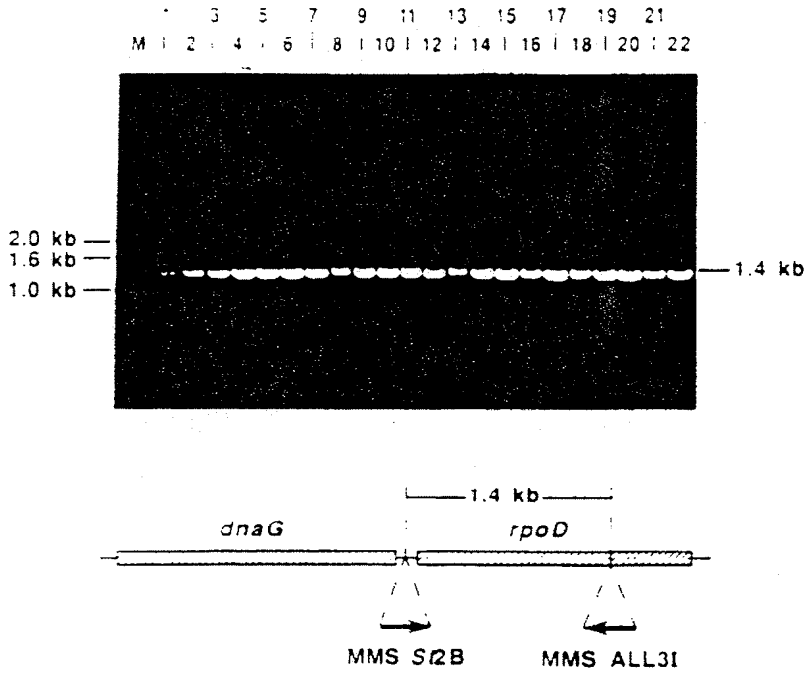

Figure 16

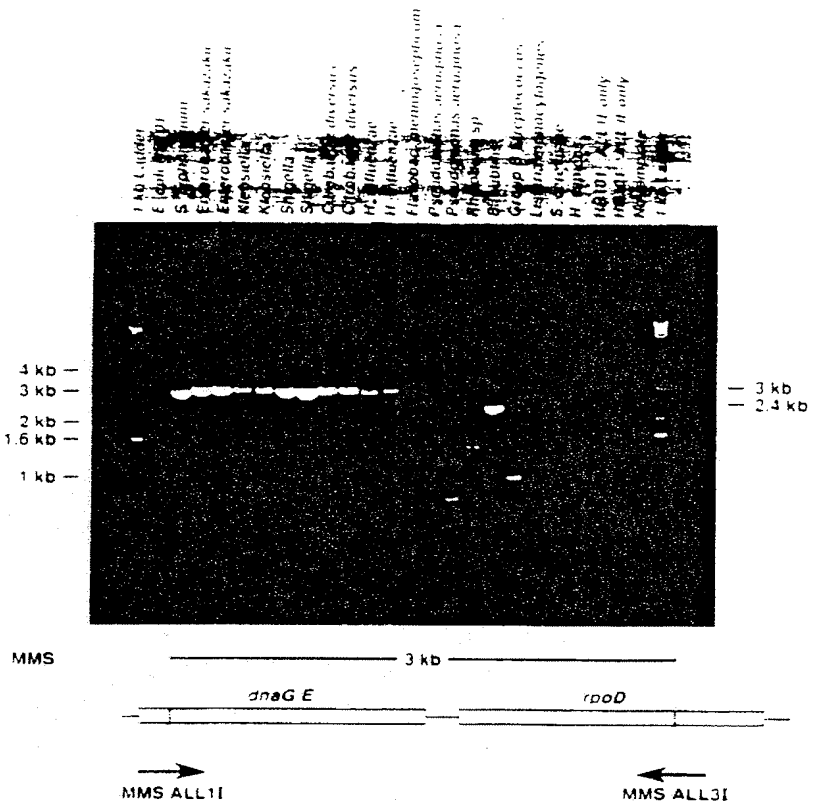

Figure 17

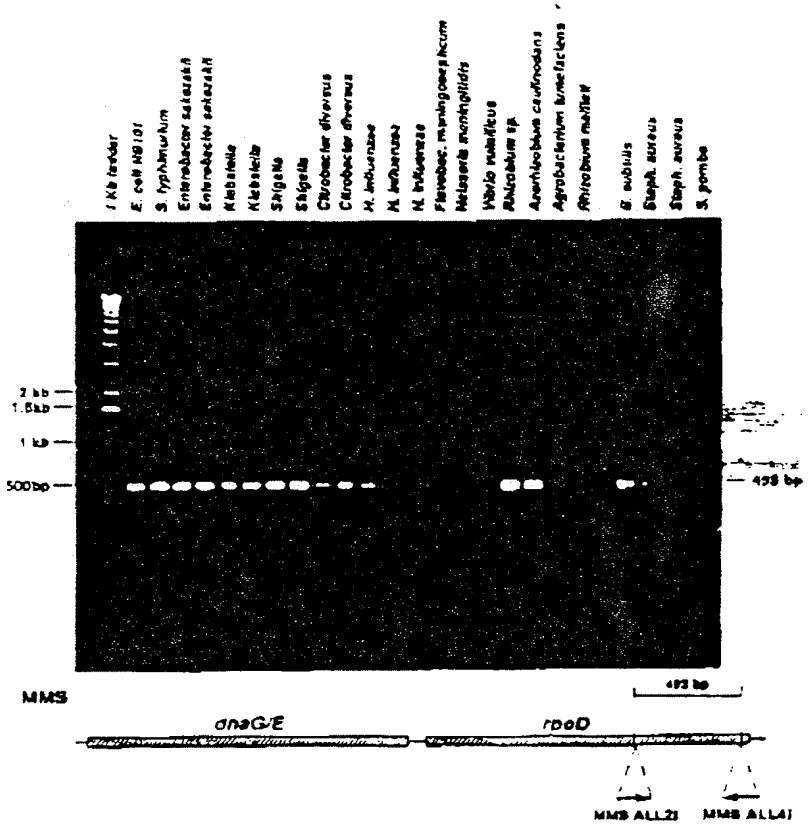

Figure 18

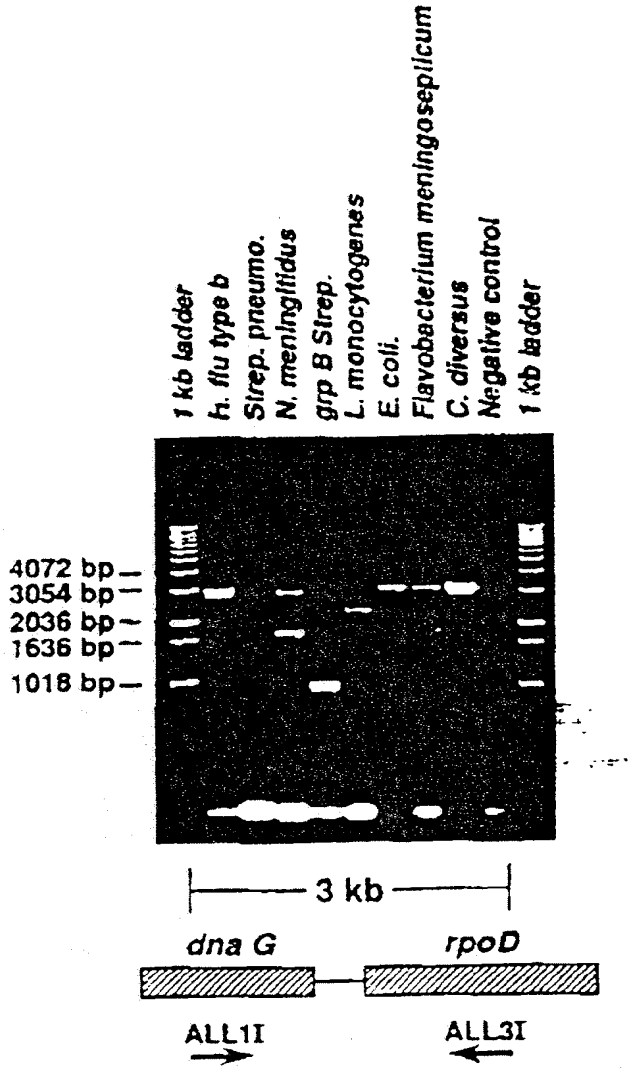

Figure 19

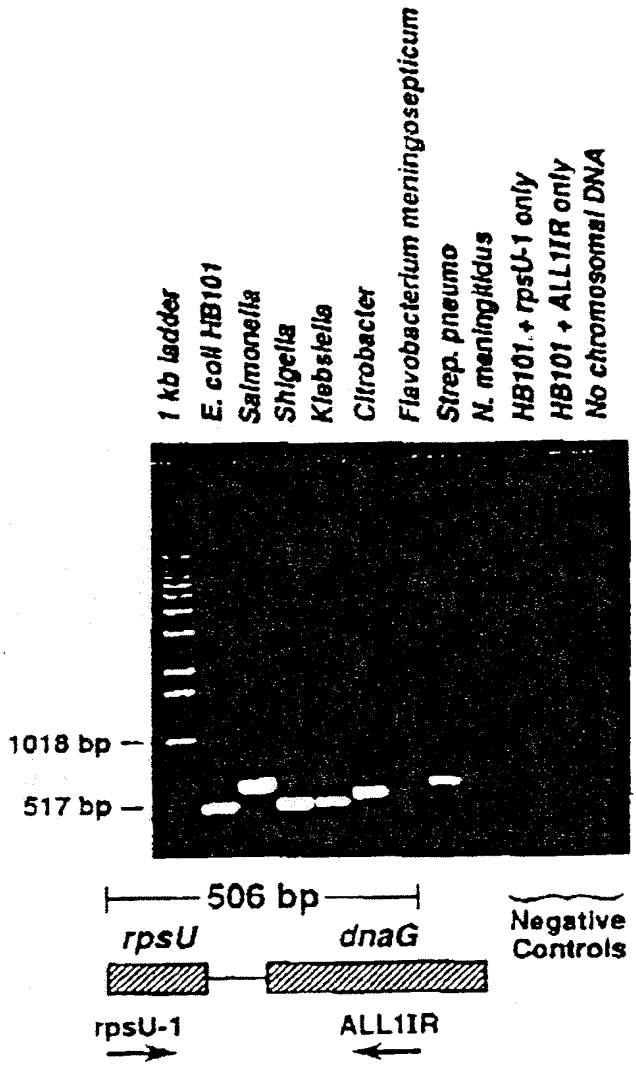

Figure 20